ABSTRACTS FOR AAGB-2014

Genomic selection in plants: Empirical results and implications for crop improvement

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Inexpensive DNA sequencing and new statistical methods are revolutionizing plant breeding. Genomic selection (GS) is the simultaneous use of genome-wide markers to increase accuracy of performance prediction for both phenotyped and unphenotyped individuals. In GS, a training population related to the breeding germplasm is genotyped with genome-wide markers and phenotyped in the target set of environments. That data is used in a prediction model to estimate breeding values of unphenotyped candidates. Design of the training population is crucial to the accuracy of prediction models and can be affected by many factors including population structure and composition. Prediction models can incorporate performance over multiple environments and assess GxE effects to identify a highly predictive subset of environments. We have developed a methodology for unbalanced datasets using genome-wide marker effects to group environments and identify outlier environments. In addition, environmental covariates can be generated using a crop model and used in a GS model to predict GxE in unobserved environments and to predict performance in climate change scenarios. Current research is focused on optimizing the training population to improve efficiency and increase prediction accuracy in terms of genotypes, experimental design and environment sampling.

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Genomic enhancement of maize for aflatoxin resistance

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Resistance to aflatoxin accumulation and ear rot caused by *Aspergillus flavus* has been studied in maize (*Zea mays* L.) using quantitative trait loci (QTL) mapping, which identified several QTL for resistance from various sources. Near isogenic lines created with these QTL validate stable phenotypic effects. Publication of the B73 reference sequence has allowed new opportunities to study the problem. The ability to identify the DNA sequence and single nucleotide polymorphisms (SNPs) within any candidate gene or gene family has allowed successful candidate gene association mapping in a panel of 300 maize inbred lines. The lines were phenotyped as testcrosses for aflatoxin levels following inoculation by *A. flavus* in replicated trials in 8 environments. Each inbred was also genotyped by sequencing (GBS) to generate 261,184 robust SNPs. Genome-wide association analysis uncovered 117 SNP-trait associations in one or more environments, and eight fell below a 10% false discovery rate (FDR, $p < 3.83 \times 10^{-7}$); these were within four uncharacterized gene sequences. All associated SNPs and their gene effects were analyzed via a gene-set enrichment procedure with information from annotated maize pathways, as cumulative effects of genes in a pathway may improve insight into biological processes contributing to resistance. Of the 298 metabolic pathways analyzed, the most significant was jasmonic acid biosynthesis (FDR = 0.001), and four other pathways were identified ($0.1 < FDR < 0.2$). Possible roles for identified pathways include biosynthesis of jasmonic acid-isoleucine, production of secondary metabolites involved in defense, purine biosynthesis, and generation of precursor metabolites and energy.

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The genome architectures of *Arachis duranensis* and *A. ipaënsis* and their comparison to the component genomes of *A. hypogaea*

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The analysis of the genomes of two exemplars of the most probable diploid ancestral species of cultivated peanut (*A. hypogaea*), *A. duranensis* (accession V 14167) and *A. ipaënsis* (accession KG 30076), is providing many insights into the component genomes of the allotetraploid *A. hypogaea*. The overall distribution of genes and repeat elements and the linkage disequilibrium along the chromosomes are now apparent. The chromosome sequences provide a fascinating viewpoint that can be interpreted in the light of information previously available through cytogenetics and linkage mapping. General differences in repeat structures of the A and B chromosomes suggest the basis of the strongly condensed centromeric bands of the A genome. Comparisons between the A and B chromosome sequences reveal the origin of the small A pair of chromosomes characteristic of the A genome. Comparisons of the diploid wild sequences with Moleculo long read data from *A. hypogaea* show high similarities. *Arachis ipaënsis* shows such remarkable similarity that it is reasonable to speculate that this accession is descended from the very same population that gave rise to cultivated peanut. These diploid genome sequences will be a key resource for assembling a genome sequence of *A. hypogaea* and can serve as highly informative proxies of the component genomes of cultivated peanut, for gene identification and marker development.

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Phenotyping *Arachis hypogaea* populations for development of genetic markers that can be used in MAS

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Phenotyping of structured populations, along with molecular genotyping, is needed for marker development in peanut. This research is essential for making the peanut genome sequence useful to breeders because it will make the connection between genes, gene markers, genetic maps, and agronomic traits in peanut. Several structured populations are available, and phenotyping efforts are ongoing. Sixteen inbred mapping populations have been created using parents that maximize genetic diversity for practical breeding objectives. First, two runner cultivars (Tifrunner and Florida-07) were selected as common parents because runner cultivars account for about 80% of the production in the U.S. Second, eight unique parents were selected to supply diversity across market classes and botanical varieties and are donors of favorable alleles for enhancing drought tolerance and resistance to most important diseases of peanut in the U.S. Phenotyping of two additional RIL populations is ongoing since these are part of the genome sequencing effort. The T population resulted from the cross of Tifrunner x GT-C20, and the S population resulted from the cross of SunOleic 97R and NC 94022. Data analysis has resulted in the identification of QTLs for resistance to several important diseases. It is anticipated that analysis of data from 2014 will result in additional QTLs for disease resistance, along with QTLs for yield and grade characteristics.

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Assessing the BAC-to-BAC assembly strategy for the *A. hypogaea* genome

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BAC-to-BAC strategy can be applied to assemble complex genomes, as breaking down the complexity using BACs will help resolving large genome size, repeat elements and heterozygous regions. For the allotetraploid *A. hypogaea*, whole genome shut-gun strategy did not result in good assembly result thus we proposed the BAC-to-BAC strategy. We first constructed two BAC libraries (~140,000 BACs) which were more than 8× of the genome. Then, we randomly selected 100 BACs to assess the assembly of BACs pools. We constructed and sequenced one sequencing library (insert size 500 bp) for BAC pools with one BAC, two BACs and four BACs. For these three BAC pooling strategies, one BAC in each pool resulted in the better assembly statistics (average scaffold N50 15 kb) but the assembled BACs were quite fragmented which would affect the whole genome assembly. To further investigate the feasibility of the BAC-to-BAC strategy, we constructed and sequenced two sequencing libraries (insert sizes: 250 bp and 500 bp) of another 384 BACs. Assembly statistics of these 384 BACs were better than the previous BACs with only one sequencing library, and this reflected that multiple insert sizes libraries should be required for BAC assembly and would work better for the whole genome assembly. The assembled BACs were also analyzed and compared to the diploid sequences. Our analysis shed lights on the complexity of the *A. hypogaea* genome and provided insights for choosing assembly strategies of this genome.

Update on the peanut genome and comparisons to other legumes

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Sequencing of tetraploid (4*x*) peanut and its diploid (2*x*) progenitors provides a framework to begin to understand the evolution and domestication of peanut as well as genomic tools to begin to introgress genes/chromosome segments from the wild into the domesticate. One of the first things we looked for in the genome sequences was transposable elements, as in most plant genomes these comprise a large fraction of the genome and can complicate annotation of genes. We developed a bioinformatics pipeline and annotated transposons in *Arachis* species. Both Class I retrotransposons and class II DNA transposons were identified in peanut, and these transposons contributed 69.0% and 74.0% of the *A. duranensis* and *A. ipaensis* genome, respectively. Notably, numerous transposons were located in or near genes and may play roles in gene regulation and evolution. In contrast to other sequenced plant genomes, we found that peanuts are enriched for long interspersed elements (LINEs). Except for the L1 group that represents the most prevalent LINEs in plants, we identified other new groups of LINEs in peanut. By combining computational and wet lab analyses, we identified a new potentially active LINE family that is widely distributed in both *Arachis* diploids and is highly expressed in *A. duranensis* and cultivated peanut, but not in *A. ipaensis.* Our results provide an important resource for the peanut community in order to annotate the genomes and to develop transposon-based markers as well as to find active transposons for peanut functional genomics.

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The Arachis transcriptome

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Escalated transcriptome data generation for *Arachis* species has enabled analyses of differential gene expression that may underlie developmental and stress response traits. As a component of the peanut genome project, transcriptome assemblies of tetraploid peanut and its diploid progenitors have aided annotation of the diploid genomes. A tetraploid assembly from the reference genotype, Tifrunner, continues to be updated as sequence is generated from additional tissues/treatments and assembly methods are refined. A developmental series for pegs and pods along with documented time points for vegetative tissues was sampled in order to construct a gene and gene expression atlas across developmental stages. Diploid progenitor genome sequences were used to guide tetraploid transcriptome assembly, greatly improving the ability to separate homeologous sequences and enabling expression bias between homeologs to be examined. Understanding interactions between duplicate genomes that affect gene expression and its phenotypic consequences will impact future breeding strategies within the cultivated tetraploid and between cultivated and synthetic tetraploids.

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Whole-transcriptome analysis of peanut tissues using next-generation sequencing: Toward an RNA-Seq atlas for NM Valencia C

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Previously, our laboratories have performed gene expression studies using EST sequencing and spotted microarrays to investigate tissue-specific gene expression and response to abiotic stress. While these studies provided valuable insight into these processes, they are constrained by sequencer throughput, expense, or require *a priori* information about gene sequence. The development of high-throughput parallel sequencing or Next-generation sequencing and its application to RNA allows for whole-transcriptome sequencing and expression analysis over a large dynamic range. We have recently used the HiSeq 2500 4th generation Illumina sequencer to sequence the whole transcriptome of leaf, stem, root, flower, and pod tissues of field-grown New Mexico Valencia C. We obtained 340 million paired-end reads which are being assembled by SOAPdenovo2, NGen, and CAP3 assemblers to generate the reference transcriptome for NM Valencia C. Our goal is create a RNA-Seq atlas of *Arachis hypogaea* to improve current gene sequence information and annotation, as well as identify genes specific to key agronomic traits. We will present our findings on genic content and tissue-specific gene expression and discuss the challenges and opportunities of unifying transcript sequence data for the peanut community.

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Transcriptome profiling of peanut developing seed with a focus on duplicate oil related pathways

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Peanut ranks among the world's most important oilseed crops, yet relative to other oilseeds there are fewer studies of oil-related biosynthetic and regulatory pathways. We used the recent UGA tetraploid transcript assembly to perform RNA-seq analyses of seeds and pods during development. Four developmental stages of seeds (R4-R7) were sampled from two peanut lines, Hanoch and PI 338338. Transcriptome data were explored with respect to genic and sub-genomic patterns of expression, globally and with respect to oil pathways. The most dynamic change in the expression was from R5 to R6 developmental stages, with 8.4% of the genes differentially expressed. The expression is significantly biased towards the A-genome in seed transcriptome, particularly at the initial pod developmental stage (R4). Co-expression analysis shows largely congruent homoeolog networks, but also homoeolog-specific divergence between subgenomes. Functional enrichment tests showed that lipid related genes were significantly represented early in seed developmental stages with an expression bias towards the B-genome. A few unique features in the oil biosynthesis pathway were found, like the contribution of the mitochondrial *Pyruvate Dehydrogenase E1* to the total pyruvate dehydrogenase expression pool; the occurrence of a single ortholog of *biotin carboxyl carrier protein* (BCCP) in peanut; and the evident contribution of the phosphatidylcholine pathway to the TAG assembly process. In general, the expression pattern of genes for enzymes involved in fatty acid synthesis was different from ESTs associated with TAG assembly suggesting that regulation is under separate control. Differences in expression of oil genes were found between the two peanut lines. For example, a complete 100% bias in expression of the *Acyl-ACP Thioesterase A* (*FATA*) gene towards the A-genome was found in PI 338338 that may explain its lower pod-filling potential. This bias may result from deletion of this gene from the genome of PI 338338. This study provides the first temporal analysis of duplicated gene expression in peanut seed and will help understanding of new aspects of oil biosynthesis in peanut.

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***Arachis* species and germplasm management**

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The genus *Arachis* has 81 named species and additional ones are currently being named. These materials are divided into nine sectional groups based on both morphology and cross compatibility relationships. The cultivated peanut (*Arachis hypogaea* L.; 2*n* = 4*x* = 40; AB genomes) is a member of section *Arachis* along with at least 29 diploid species (2*n* = 2*x* = 20). Although peanut species have been collected in South America for more than 100 years, systematic collections began during the early 1960's by Drs. A. Krapovickas and W.C. Gregory. Their work has been continued by researchers in Brazil, Argentina, the U.S., and India. More than 3000 species accessions were identified by the year 2000, of which about 1000 exist in germplasm collections. Although peanut collection is continuing in Bolivia, Brazil and Argentina and new accessions and species are being added to their collections, political decisions have prevented germplasm exchange from several important South American countries to the U.S. or Asia since 2001. Several large germplasm collections do exist, however, in the U.S., Brazil, Argentina, India, and China. Many of the species are difficult to maintain because they produce few seeds under cultivation and about 1/3 of the accessions are maintained vegetatively. A large number of accessions have been evaluated for many disease and insect resistances as well as for quality traits, but significant work remains to be completed before the full potential for utilization will be understood. Germplasm lines and cultivars have been released with *Arachis* species in their pedigrees, several of which are both highly productive and disease resistant.

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Roadmap of the USDA peanut germplasm collection: Past, present and future direction

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The USDA ARS PGRCU maintains the second largest *Arachis* germplasm collection in the world with 9,321 cultivated and 655 wild entries. In the last twenty years, USA germplasm has been provided to over 52 countries around the world for research and breeding purposes. This collection has proven to be a useful resource to mine for traits to improve peanuts by identifying sources of resistance for root knot nematode, leaf spot, tomato spotted wilt virus (TSWV), preharvest aflatoxin, and Sclerotinia. Progress has been made in the last few years to improve the overall state of this valuable collection. The number of unavailable lines in this collection has dropped by 20% in three years which allows researchers and breeders access to the germplasm needed for their programs. Germination data on all the accessions has now been collected allowing more informed decisions on regeneration priorities. Storage conditions have been improved by building additional -18°C cold storage space which should improve the overall longevity of the seeds in storage. Genetic gaps have been filled by exchanging material with national and international breeders, as well as genebanks around the world, which has significantly increased the number of cultivated and wild species in the USA collection. Intensive phenotyping and genotyping work is being collected on important collections such as the core and mini core to provide information to the breeders in order to make improved selections for their breeding programs. All of these changes are helping to strengthen the state of the collection and its overall utilization.

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An overview on peanut germplasm collection, evaluation, and utilization in China

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Peanut (*Arachis hypogaea* L.) is an important source of vegetable oil and protein worldwide, with China being the largest producer during the past two decades. Genetic enhancement has been crucial in peanut industry development in China and many other countries. Systematic collection and preservation of peanut germplasm in China was initiated in early 1950s. To date, a total of 8,439 accessions of *A. hypogaea* covering all the six botanical types have been collected and preserved under coordination of OCRI-CAAS. Compared to large peanut collections in other countries, relatively more diversified Dragon type (equal to var. *hirsuta*) landraces have been collected and maintained in China as varieties of this type were most extensively cultivated until the early 20th century. A core collection of 576 accessions, a primary mini core of 298 accessions, and a mini-mini core of 99 accessions have been selected. Differences between the Chinese and ICRISAT mini core collections were comparatively studied. Analysis of SSR markers revealed a higher level of genetic diversity in the Chinese mini core than in the ICRISAT mini core. Elite lines with special traits such as high oil content, resistance to foliar diseases, bacterial wilt, pod rot, aflatoxin, and nematodes have been identified from the Chinese and ICRISAT mini core collections. The Chinese peanut collection is believed to be important sources for bacterial wilt and pod rot resistance, drought tolerance, and high oil content. Association analysis has identified certain molecular markers related with key traits. The identified elite genotypes have been extensively utilized in production and/or in breeding programs. About 40 peanut landraces, mostly belonging to Virginia and Spanish types, have been successfully used as direct or indirect parents of the released improved

cultivars (more than 200) during the past five decades. Fuhuasheng and Shitouqi were most extensively involved in breeding as direct or indirect parents in 161 and 52 released cultivars respectively, indicating their unique value in breeding. The improved varieties have played an important role in enhancing peanut productivity and quality.

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Species, genomes and diversification in section Arachis

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In the last 10 years, botanical collections of *Arachis* species have been intensified in Bolivia. Several new species have been discovered with some very interesting characters, and the range of geographic distribution was expanded for many of known taxa. In the same period the species of section Arachis were re-arranged in six different genomes and in three karyotypic subgroups. In this study we analyzed the distribution of the Arachis section species and the variability of chloroplast sequences (*trn*T–S and *trn*T–Y) in order to understand the dispersal pathways and to shed light on the evolutionary history of the section. The range distribution of the species showed a biogeographic segregation of most of the genome and karyotype groups. Most of them were associated to different biogeographic regions and river basins but the chloroplast haplotypes recovered from the species did not. The major diversity of haplotypes was concentrated in the Chiquitanía region, in the San Ignacio Planalto. Two central haplotypes were recognized, one of them for the A genome species and the other for the B, D, K, F and G genomes. Both central haplotypes were widely distributed, covering most of the species range. The remaining haplotypes (19) were more restricted or specific to particular populations. The patterns of species and haplotype distributions, together with the analyses of main paleochannels in central South America, suggests that hydrochory may have played a key role in long distance dispersal and establishment of founders in allopatry. Genome differentiation may have occurred in different river basins during Pliocene, while speciation within each genome may have occurred also in isolation with incomplete linage sorting for the markers analyzed.

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Characterization of Gregory x *Arachis diogoi* (GK 10602; PI276235) interspecific hybrid population

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Arachis diogoi (GK 10602: PI 276235) is a diploid (2*n*=2*x*=20) wild species in section *Arachis* and is cross-compatible with the cultivated species, *A. hypogaea* L. (2*n*=4*x*=40). *Arachis diogoi* exhibits high levels of resistance to leaf spots (early and late leaf spot), rust and *Tomato spotted wilt virus (*TSWV). The triploid-hexaploid introgression pathway by direct hybridization of *A. diogoi* as the pollen parent with *A. hypogaea* cv. Gregory as the seed parent, was used to develop the interspecific hybrid population. The original F_1 hybrid was completely sterile and was confirmed by cytological analysis as a triploid (2*n*=3*x*=30). Vegetative cuttings from the hybrid were treated with 0.2% colchicine solution for 8 h, cut ends rinsed thoroughly with water, treated with rooting hormone, planted in the greenhouse on a sand bed under shade and misted. Ploidy of the resulting plants was determined by cytological analysis and one hexaploid (2*n*=6*x*=60) plant was recovered. The triploid and hexaploid plants were robust and were extremely prostrate in habit similar to that of the wild species. The hexaploid and its progeny were allowed to self-pollinate for 11 generations without any artificial selection. Most plants resembled *A.*

hypogaea phenotypically, for plant habit, leaf, flower, pod, and seed size providing the current interspecific hybrid population. A random sample (n= 17) of greenhouse grown hybrid plants along with both parents by flow cytometric analysis indicated that the average genome size of the hybrids was 6.67 pg (range 6.51-6.91) versus 6.61 for Gregory and 3.59 for *A. diogoi*, suggesting that the hybrids are tetraploid. A preliminary SSR marker analysis of hybrids showed DNA bands unique to both parents in the hybrids validating the hybridity and evidence for the presence of *A. diogoi* DNA in the hybrids. Additionally, a limited (*n*=25 each) evaluation of the hybrid plants along with both parental samples for thrips feeding, infection and transmission of the virus assays indicated that the hybrid plants had reduced TSWV accumulation, almost 10-fold lower as compared to Gregory. During the 2014 crop season in Florence, SC, late-season field evaluation of hybrid progenies showed that about 64% plants had no TSWV symptoms. In the same study, Gregory had 56% TSWV infected plants. Additional laboratory and greenhouse assays are being conducted and results will be discussed.

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Developing an *in vitro* method to assess aflatoxin biosynthesis suppression in *Aspergillus flavus* through RNAi technologies

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The soil-inhabitant fungus *Aspergillus flavus* is consistently associated with agronomical fields, where it promptly colonizes important crops such as corn (*Zea mays*) and peanuts (*Arachis hypogaea*). The consumption of *A. flavus*-contaminated food grains poses a potential threat for human and animal health and overall food security. Generally *A. flavus* synthesizes two major mycotoxins: aflatoxin B1, a polyketide-derived carcinogenic toxin with hepatotoxic and immunosuppressive effects in humans, and cyclopiazonic acid, an indole-tetramic acid neurotoxin that affects liver, kidney, and gastrointestinal tract in animals. It has been estimated that more than 4.5 billion people around the world are at high risk to the adverse effects of aflatoxins exposure. Therefore, the control of aflatoxin contamination in key staples requires comprehensive and sound approaches. RNA interference (RNAi) technology, which is based upon a natural process to suppress the activity of specific genes, has the potential to prevent mycotoxins accumulation in crops. In our study, we have developed an *in vitro* system to evaluate the effectiveness of RNAi-based molecular constructs in suppressing aflatoxin biosynthesis as preliminary screening before using these constructs in plants.

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Using RNAi technology against mycotoxin-producing *Aspergillus* and *Fusarium* species

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RNA interference (RNAi), a natural biological process, provides gene regulation and defense in humans, plants, fungi, insects, and many organisms. Now it is being studied and exploited to understand gene expression, ameliorate human diseases, and provide resistance to plant viruses. We are extending it to provide resistance to fungi to control mycotoxin production. Venganza is the Spanish word for revenge. We identify genes from fungal pathogens that are essential for them to live, reproduce, cause disease, and produce mycotoxins. Those essential genes are transformed into plants in a form recognized by the plant as foreign, causing the plant's ribonuclease defense mechanism to cut the transcript into small pieces, small-interfering RNAs (siRNAs). These siRNAs are consumed by feeding fungi, creating double-stranded regions along the mRNA transcripts of those essential genes, causing their destruction and silencing within the fungus, inactivating or killing it, making the plant resistant. Therefore, plants get revenge on fungi using the fungus' own genes. This provides plant breeders a novel source of resistance genes, from the fungi themselves. Fungal secondary metabolic pathways producing mycotoxins are

extensively characterized. Their crucial steps are ideal targets for disruption by RNAi to prevent synthesis of these hazardous compounds. Proof of concept for RNAi, provided by McDonald, et al. 2005, demonstrated that transformation of *Aspergillus flavus*, *A. parasiticus*, and *Fusarium graminearum* with inverted repeats of mycotoxin regulatory gene sequences suppressed mycotoxin production in all three species. Cooperative with CIMMYT and supported by USAID, we are targeting both *Aspergillus* and *Fusarium* to control the diseases and mycotoxins they produce in maize and wheat.

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Transgenic interventions for host-plant resistance for *Aspergillus flavus* infection and aflatoxin contamination in peanut

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Aflatoxin contamination due to *Aspergillus flavus* infection is a serious threat to the safety and marketability of peanuts worldwide. Host-plant resistance is a highly desirable preventive strategy to reduce pre-harvest aflatoxin contamination in peanut. Developing crop resistance to aflatoxin remains a challenge in view of the complexity of host-soil-environment interactions required for *A. flavus* growth and toxin production. Since both biotic and abiotic factors are known to influence *A. flavus* infection and subsequent aflatoxin contamination, the burden of aflatoxin contamination in the semi-arid regions has been exacerbated by the prevailing weather conditions. Conventional breeding methods for enhancing host-plant resistance for aflatoxin contamination have offered limited success in peanut due to the complexities involved, thereby rendering the majority of elite breeding lines largely susceptible to aflatoxin contamination. Developing transgenics using novel biotechnological tools is potentially a more reliable method in aflatoxin resistance. Efforts are under way at ICRISAT to develop transgenic peanut for resistance to *A. flavus* infection and aflatoxin contamination using various antifungal genes, synthetic peptides, defensins, lipoxygenases, and dispersive proteins. To find a sustainable solution to this problem, there has been a critical focus on identifying and improving knowledge of host-plant resistance factors to aflatoxin accumulation, besides exploring host-induced gene silencing (HIGS) as a potential strategy. This can provide scope for practical solutions to the pre-harvest fungal infection and aflatoxin contamination in peanuts. Biotechnological approaches for minimizing pre-harvest infection and aflatoxin contamination in peanut will be discussed.

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Combining genomic approaches to understand genetic control of aflatoxin contamination in peanut

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Aflatoxin contamination in peanut is more prevalent under rain-fed conditions, making produce unfit for human and animal consumption and therefore adversely affecting the international trade. Although the losses in yield and quality due to aflatoxin contamination are higher than realized, there are limited resistance resources available to use in aflatoxin resistance breeding. Hence, attempts are being made to use different genomic approaches to tackle this challenge. In the first approach, transcriptome sequencing of resistant (J 11, ICGV 91278) and susceptible (JL 24) genotypes has been carried out to study the resistance to *in-vitro* and pre-harvest colonization of seeds by *Aspergillus flavus* (strain AF-

11F). The sequencing of transcripts generated 64 to 94 million reads using Illumina HiSeq 2000 platform in case of *in-vitro* seed colonization. The differentially expressed reads will be analyzed to identify the candidate resistant genes and understanding the underlying mechanisms. In a second approach, peanut 'reference set' was screened for aflatoxin contamination across four environments at Niger and the analysis is underway to identify marker-trait associations for aflatoxin contamination. As there are no bi-parental populations available for aflatoxin resistance in peanut, multi-parent advanced generation intercross (MAGIC) populations are being developed for aflatoxin resistance along with other important agronomic traits as the third approach. The comprehensive efforts to identify marker-trait associations and the transcripts responsible for varying resistance to aflatoxin will be a useful resource to address this serious constraint.

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Progress on genetic linkage maps, traits/QTLs, and utilization in two recombinant inbred line populations of peanuts (*Arachis hypogaea* L.)

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Peanut, a highly nutritional crop, is used in edible products or crushed for cooking oil, and is susceptible to a range of diseases, including *Tomato spotted wilt virus* (TSWV), early and late leaf spot (ELS and LLS). Losses in productivity and quality are also attributable to environmental stresses and food safety issues. A promising solution is the use of quantitative trait loci (QTL) mapping of disease resistance and other agronomic traits for use in cultivar development. Two recombinant inbred line (RIL) populations have been developed for genetic mapping and QTL study, one derived from the cross SunOleic 97R × NC94022 (referred as S-population) and another from Tifrunner × GT-C20 (referred as T-population) with 352 and 248 individuals, respectively. These two populations were used in QTL analysis for oil quality including total oil content and fatty acid composition, disease resistance including TSWV, ELS and LLS, and other traits from 2009 to 2013. The first generation maps were developed with 172 and 239 marker loci and two QTLs were identified for TSWV resistance with 36% and 13% phenotypic variance explained (PVE) for the S and T population, respectively. The second generation maps were constructed with 206 and 377 marker loci. Major QTLs were identified for oil content and eight fatty acid compositions. These maps were further saturated and the current third generation maps contain 248 and 426 marker loci. Disease resistance QTL regions with high PVE were identified for ELS, LLS and TSWV resistance. Interestingly, several gene-rich linkage groups have been identified and used for comparison with diploid pseudomolecules in order to identify specific genes and "gene-rich" chromosomes. Furthermore, sequence data for 141 and 118 RILs from the S and T population are being generated under the international peanut genome sequencing project, respectively.

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Prospects for a SNP chip in cultivated Peanut (*Arachis hypogaea*) utilizing the leaf transcriptome

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High throughput next generation sequence-based genotyping and SNP detection opens the door for emerging genomics methods such as genome-wide association analysis and genomic selection-based breeding. High density SNP markers allow easy identification of tightly linked functional markers to important disease resistance QTL. Recently we have developed a bioinformatics pipeline (SWEEP) which utilizes the diploid progenitor genome sequences to call SNPs in tetraploid peanut. Utilizing the newly released diploid progenitor genomes (*Arachis duranensis* and *A. ipaensis*), we use a sliding window approach that visits each called SNP and looks for a neighboring homeologous haplotype, using this haplotype as a guide to decide if the SNP is allelic. In a pilot study, we were able to confirm by Sanger Sequencing 81% of a randomly sampled 25 SNP loci. In the present study we sequenced the leaf transcriptome of six genotypes that are parents of a set of 16 RIL populations being phenotyped for abiotic and biotic stress tolerance/resistance; Florida07, C76-16, NC3033, SPT06-6, New Mexico Valencia A, and Tifrunner. After using our SWEEP algorithm, we identified 35,864 and 34,540 high quality SNPs relative to the A and B genome respectively. Pairwise polymorphism ranges between 13,738 – 19,348 for A SNPs and 13,578 – 19,211 for B SNPs. These SNPs represent a strong data set from which to select a gene-associated SNP chip for peanut.

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The use of the diploid *Arachis* genomes to aid introgression of wild segments into peanut

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Diseases are important reducers of peanut (*Arachis hypogaea*) yield. Wild species generally harbor greater levels of resistance and even apparent immunity. Genomic regions conferring resistance to foliar fungal diseases and root knot nematodes have been identified in populations involving the wild progenitors of peanut, *A. ipaënsis* and *A duranensis* crossed with species that harbor resistances to these pests, including *A. magna* and *A stenosperma.* For introgression of these genomic regions, induced allotetraploids were produced: [*A. gregoryi* × *A stenosperma*]^{4x}, [*A. magna* × *A. stenosperma*]^{4x} and [*A. batizocoi* × *A. stenosperma*]^{4x} and used to cross with elite varieties in Brazil and in the USA. Molecular markers residing in the vicinity of genomic regions controlling disease resistances were developed using the diploid peanut genome sequences to develop tightly linked, easy-to-use microsatellite and SNP markers for foreground selection. In addition to this, SNP markers were developed for monitoring introgression of wild species DNA into cultivated peanut on a genome scale by calling polymorphisms between the wild species and *A. duranensis* and *A. ipaënsis* as proxies for the cultivated peanut component genomes. In this way, each genome component was dissected for marker development. These examples are among the first markers developed using the diploid peanut genomes.

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Interspecific resolutive mapping populations for marker/trait association in peanut

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Cultivated peanut is a recent allotetraploid with limited genetic diversity at the DNA level. Consequently, molecular breeding efforts have long been impeded 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 the framework of the Generation Challenge Programme, CIRAD, ISRA and EMBRAPA have collaborated for developing resolutive interspecific QTL mapping populations (AB-QTL and CSSL) using the synthetic tetraploid (*A. ipaensis* × *A. duranensis*)^{4x} as the wild donor. The CSSL population is of particular interest. It has been developed to represent 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. Such a population allows breeders to access novel genetic variations coming from the wild species in a way that can be easily usable because of the reduction of the negative effects resulting from the interactions between donor alleles. For example, the CSSL population shed new light on the complex inheritance of the peanut growth habit. Moreover, CSSLs are a useful starting point for breeding. As the lines are permanent, they have been shared with different partners, phenotyped for various traits in multiple environments and used in crosses for pyramiding the wild segments containing QTL. The recent release of the peanut diploid genomes opens new avenues for a comprehensive characterization of this genetic resource.

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Research progress on peanut genetic trait mapping in China

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QTL mapping research in China mainly focused on agronomic traits, quality traits, and disease resistance to Web Blotch, Bacteria Wilt and *Aspergillus flavus.* HAAS identified 44 SSR markers linked to loci controlling the content of protein, fat and oleic acid by association analysis using 136 peanut cultivars from different provinces in China. Recently, 58 QTLs related to 14 traits of peanut yield and quality in three different environments were detected using RILs derived from Baisha1016 × *A. monticola,*. OCRI located QTLs related to BW resistance on linkage group1, 4 and 14 using a RIL population. In an association study, using a set of peanut mini-core collection of 146 varieties, a total of 55 loci were identified to be associated with 12 traits. GAAS identified one SSR marker linked to peanut testa color. They also reported the study on 12 peanut traits based on their constructed linkage maps. A total of 42 QTLs were detected. Besides the efforts exerted on QTL mapping of peanut in China for the past decade, application of marker assisted selection remains a great challenge. So far, MABC (marker assisted backcross) for oleic acid content was one of the few available MAS applications in China due to the lack of markers closely linked with traits of interest and the lack of a high throughput marker screening technique. In the future, NAM (nested association mapping) and MAGIC (multi-parent advanced generation intercross) populations are promising for QTLs mapping. SNP markers would benefit breeding program with the development of GBS (genotyping by sequencing) and high efficiency technique for screening the SNP markers being popularized.

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Using PeanutBase to explore the *Arachis* genomes and peanut genetic information.

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We will describe the main features and use of the PeanutBase website (http://peanutbase.org), including genome browsers, genetic map viewers, sequence search tools, a database of traits and QTLs, and marker-assisted selection pages with detailed information about markers and accessions for some high-value peanut traits. This talk will also describe some next steps for PeanutBase, including ways for the peanut breeding and research community to contribute important information and to shape this resource to meet their needs. A special focus of the project has been to collect and integrate trait and map data from many peanut research papers, and we would like to engage researchers to help extend these information resources. Following the presentation we will be available to help with hands-on use of the website for people who would like to explore using their own laptop computers. PeanutBase was started in April 2013 at Iowa State University with funding from The Peanut Foundation and in-kind contributions from USDA-ARS.

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Integrated Breeding Platform: A novel set of tools and services to support breeding programs

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Many national programs in developing countries are not using basic data management and analytical tools for crop improvement, which leads to sub-optimal breeding decisions. Compounded over years, and escalated by limited human resources and insufficient field infrastructure, the low rate of gain in crop improvement is a substantial drag on overall crop productivity in the developing world. The Integrated Breeding Platform (IBP –www.integratedbreeding.net), developed by a broad set of partners under the coordination of the CGIAR Generation Challenge Program, has developed a comprehensive suite of tools and services to support routine crop breeding program activities. Improved data management and analysis will increase breeding efficiency – particularly by facilitating the integration of modern breeding approaches such as the use of molecular markers – thus reducing the time and resources required to produce more resilient crops with better yield under local target conditions. The IBP also provides the intensive professional support and mentoring needed for adoption of these best practices through a structured capacity building approach and facilitated access to breeding services such as genotyping. Dissemination of IBP tools and practices and support to local users will be ensured by Regional Hubs established in collaboration with key partner institutions. The IBP is now entering into a promotion and deployment phase aiming at increasing the efficiency of up to 500 plant breeding programs in the developing world over the next 5 years, both in the public and private sectors, with ultimately attendant improvement in livelihoods for smallholder farm families who depend upon food security crops.

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Challenges and Research Opportunities for Peanut Production and Pest Management in the US

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Peanut production systems in the United States are diverse depending on geography, market opportunities, pest complexes, and resource limitations. Scientists in traditional breeding program, those focusing on wild species integration and utilization of genomics work closely to provide farmers with improved genetics that form the backbone of modern peanut-production systems. Defining interactions of environmental and edaphic conditions with varieties has long been an important element of research both in the US and worldwide. In addition to growers managing genetics under production constraints and opportunities in each region and farming operations within a region, they also consider ramifications of business and marketing decisions and the role of government policy in their farming decisions. While diseases, insects, and weeds offer many challenges in optimizing yield, specific pathogens or pest species within these broad categories often vary by region in terms of incidence and severity and subsequent economic impact. While improvement in genetics by the industry continue to address economically-damaging pests and pest complexes, improvements in areas of drought resistance, seedling vigor associated with environmental and edaphic conditions, and greater flexibility in genetic resources associated with crop development are needed in any areas of the US. Competition between farming and urban interests for water, land and other resources will continue to be a prominent issue and will require greater water use efficiency of cultivars and optimization of yield potential. Extremes in weather patterns aligned with climate change also will require alternatives for growers in order to maintain yield and market grades under unpredictable conditions, especially as components of the US industry shift to high oleic cultivars. Restrictions on pesticide use and the need to change production practices to protect natural resources will increase in the future. Availability of cultivars that offer a complete package of pest resistance, flexibility in planting, and water-use efficiency will be important in the future for growers in the US. In addition to these components of production systems, improvements in genetics relative to aflatoxin management and other food-safety challenges will be increasingly important. As improvements in genetics in the US increase with respect to many issues, the technologies associated with those improvements will also contribute to peanut production worldwide.

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Peanut improvement for drought prone environments of Sub-Saharan Africa: Did we get good old agronomy right?

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Drought stress limits peanut productivity in Sub-Saharan Africa, but drought scenarios vary across time and geographical scales. The extent of this variation and, more importantly, the consequence on crop yields has not been described. Also, over the past decades, the peanut plant type cultivated in Africa has

evolved from predominantly spreading types of medium to long duration with quite profuse foliage to now mostly erect types of early duration with much smaller foliage. However, the recommended seeding rate of 60 kg seed ha^{-1} has remained unchanged with this change in plant characteristics. It is difficult to determine experimentally whether the past recommended peanut growth pattern and sowing practices (short duration cultivars, 60kg ha^{-1} seed rate) are still appropriate, as they involve both genetic and agronomic factors. A crop simulation model developed for peanut (SSM, Simple Simulation Model) was used to explore what might now be optimal practices. First, the robustness of the model was demonstrated across 16 different trials carried out in Niger and India, in which simulated yields fell within 16% of the observed across a large range of yields. The SSM-peanut model was then used to test the effect on yield in a typical Spanish type of sowing density varying between 20 plant m^{-2} (recommended seed rate) and 40 plant m^{-2}, and of altering the crop duration. A grid of weather data with a one degree latitude/longitude resolution was generated by Marksim at 475 locations (50 years per location), 233 in East-Southern Africa and 242 in West Central Africa. A density of 40 plant m^{-2} increased yield by 20 to 30 % across locations. However, reducing crop duration by 10 biological days for a Spanish type reduced grain yield by about 10-20% in West Africa. Using the optimal sowing rates and a regular Spanish type, the extent of drought effects on yield were geographically represented in the grid, pointing to substantial drought only in latitude above 13 degrees latitude in WCA, while drought decreased yield by 30-60% in most locations of ESA. Finally, simulations were done to give insights on some potential genetic traits leading to future peanut improvements within the context of optimal agronomic practices.

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Diseases of Peanut

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Diseases caused by several types of pathogens represent major limiting factors for peanut production worldwide, causing both direct losses in yield or quality and reduced profitability through costs associated with control. Disease resistance is a major focus of most breeding programs, and a major focus of programs involved with genetic mapping and marker development. This presentation will include an overview of the major diseases of peanut, the damage they cause, and methods used to control them. It will also address some of the methods for screening for resistance, especially those that can be used for large numbers of genotypes such as are necessary for mapping, and the attributes and shortcomings of those methods.

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Considerations for Marker-assisted selection in peanut

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Marker-assisted selection (MAS) offers considerable promise but requires careful planning. Among the first DNA markers used for peanut improvement were wild species-derived alleles for nematode resistance, now being combined with the high-oleic trait. These are screened as qualitative traits. These efforts have contributed to release of nematode-resistant cultivars, but are simple compared to future needs. Most traits are quantitative, controlled by multiple genes. Some QTLs (quantitative trait loci) have been developed for resistance to leaf spots and rust, tolerance to drought stress, and agronomic and kernel traits. MAS will require pyramiding QTLs, and can combine genes with different functions, reduce linkage drag, and develop more-durable pest resistance. Populations for marker work are typically F_2's, backcrosses, and RILs, including 16 CAPS RIL populations developed for GWAS (genome-wide association studies). Markers need to be validated when used in new genetic backgrounds. Use of wild species, plant introductions, and mini-core accessions can provide additional and stronger QTLs. Sufficiently-large populations are needed for combining QTLs. Linkages among favorable and unfavorable alleles need to be broken. Recurrent selection can break up conserved linkage blocks; MAGIC populations can for multiple parents. Scoring in the F_2, F_3, or backcross generations permits earlier selection of quantitative traits, and has the potential to cut cultivar development time substantially, but will require larger populations earlier. Although genotyping costs are declining, breeding programs will face choices of resource allocation among traits, markers, crosses, and field testing. Finally, potential cultivars will need to be field tested to verify phenotypes.

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Changes in yield potential, grade, and seed weight among University of Florida advanced breeding lines and recently released cultivars

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Two major goals in peanut breeding are improving pod yield and the Total Sound Mature Kernel percentage (TSMK). Seed size is another important characteristic of interest in peanut breeding. A 'head-to-head' statistical analysis was performed in which a group of 20 University of Florida (UF) peanut lines made up of four recently released UF cultivars, nine breeding lines tested in the Uniform Peanut Performance Tests (UPPT) from 2011-2014, and five advanced breeding lines were compared to Georgia-06G and to Florida-07. Comparisons were made when the UF line/cultivar was in the same test as the Georiga-06G or Florida-07. Comparisons varied from a minimum of three tests over two years to a maximum of 54 tests over six years for comparisons with Georgia-06G and a minimum of one test in a single year (two lines) to a maximum of 50 tests over seven years for Florida-07 comparisons. Compared to Georgia-06G, eight lines had higher pod yield, one had lower pod yield and the remaining eleven had similar pod yield ($p<0.05$). Compared to Florida-07, one line yielded less, six yielded more and eleven were similar ($p<0.05$; two advanced breeding lines had insufficient data for statistical analysis). Six lines had greater seed weight (g per 100 seeds) than Florida-07, one had lower and eleven had similar seed weight ($p<0.05$). Compared to Georgia-06G, eight lines had lower seed weight, five had greater seed weight and seven had similar seed weight ($p<0.05$). Sixteen lines had greater TSMK than Florida-07, and two were similar to Florida-07 ($p<0.05$). Two lines had TSMK greater than Georgia-06, six had lower TSMK and twelve had similar TSMK to Georgia-06G. These results support the conclusion that yield potential of several new lines is superior to Georgia-06G and Florida-07. Seed size and TSMK of future UF releases will likely be similar to Georgia-06G where as TSMK will likely be superior to TSMK of Florida-07.

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Towards deploying genomic selection for improving complex traits in peanut

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Marker-assisted backcrossing (MABC) is an effective approach for improving qualitative traits and has been successfully used to develop improved lines for rust resistance and high oleate trait in peanut. Further efforts are underway to pyramid genomic regions for multiple qualitative traits (rust resistance, late leaf spot resistance and high oleate trait) through MABC in existing cultivars. However, genomic selection (GS) has emerged as the most promising breeding approach for improving complex traits. GS can capture small-effect QTLs and develop superior lines with multiple traits. With an objective to improve complex traits like yield under drought stress, the GS approach has been initiated in peanut. In this context, the 'minicore collection' with 184 genotypes has already been evaluated for three important agronomic traits (days to flowering, seed weight and pod yield). Therefore this collection has been genotyped with 15,360 diversity array technology (DArT) features. A total of six GS models (Ridge Regression-BLUP, Bayesian LASSO, Random Forest Regression, Kinship GAUSS, BayesCπ and BayesB) were tested on the phenotypic and genotypic data for estimation of correlation and cross-validation values. As a result, two best performing GS models namely Ridge Regression-BLUP and Bayesian LASSO have been identified for predicting genomic estimated breeding values (GEBVs). Furthermore, a training population comprising of 310 elite genotypes has also been constituted and genotyped with 15,360 DArT/DArTseq features. The population is being phenotyped for several agronomically important traits. The genotypic and phenotypic data will be used to define the appropriate GS model for deploying GS in peanut breeding.

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Cloning and expression analysis of *FUSCA3* gene in peanut (*Arachis hypogaea* L.)

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FUSCA3 encodes a protein containing a B3 domain. In this article, a *FUSCA3* gene was cloned from the seed of peanut (*Arachis hypogaea* L. cultivar Huayu17) using RT-PCR and was designated as *AhFUSCA3*. The sequence of *AhFUSCA3* cDNA was 1532 bp, and its open reading frame was 1134 bp, and its genomic sequence was 3892 bp. Bioinformatic analysis showed that *AhFUSCA3* was composed of 6 exons and 5 introns with typical GT-AG characteristic in comparison of its sequences of genomic DNA and cDNA by Splign in NCBI. A peptide of 378 amino acid residues with protein molecular weight of 42.3 kD and isoelectric point (pI) of 5.58 were deduced from AhFUSCA3. Its protein was predicted to be located in cell nucleus, containing the conserved B3 domain. Multiple sequence alignments and phylogenetic analysis of FUSCA3 proteins indicated *AhFUSCA3* was most similar with FUSCA3 from *Glycine max*, *Phaseolus vulgaris*, *Cicer arietinum* and *Medicago truncatula*. The results of Real time RT-PCR showed that the expression of *AhFUSCA3* gradually increases in abundance during the initial stages of pod or seed development, but then showed a decrease in abundance at late stages. These results suggested that *AhFUSCA3* may play an important role in the process of peanut embryogenesis and peanut triacylglycerol synthesis.

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Mapping quantitative trait loci for resistance to early leaf spot of groundnut

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Groundnut (*Arachis hypogaea* L.) is an important global oilseed crop and a major source of protein and vitamins in many rural areas of Africa. In Zambia, the production of groundnut is limited by several factors, among which Early Leaf Spot (ELS) caused by *Cercospora arachidicola* Hori, is a major destructive disease. Development of resistant varieties to ELS remains the most effective disease management strategy. The objective of this study was to map quantitative trait loci (QTL) associated with resistance to *C. arachidicola* as the first step towards the deployment of marker-assisted breeding for groundnut in Zambia. Parental genotypes (Robut 33-1 susceptible and ICGV-SM 95714 resistant) were screened using 394 Simple Sequence Repeat (SSR) markers. Of these 82 (about 21%) were polymorphic. All polymorphic markers were used to screen 113 F_8 recombinant inbred line (RIL) populations alongside their parents. Phenotyping of the RIL populations was carried out under field conditions supplemented by irrigation and utilizing diseased debris as primary inoculum. Inclusive composite interval mapping (ICIM) analysis identified one major and two minor QTLs associated with resistance to ELS. Two QTLs were mapped on linkage group 3 with phenotypic variation explained (PVE) by the marker values of 37.91% (LOD 15.73) and 7.98% (LOD 3.5) and additive effects of 25.64 and -11.14 respectively. The third QTL was mapped on linkage group 4 with a PVE value of 12.31% (LOD 5.5) and additive effect of 12.92. All the mapped QTLs were less than 5 cM from the nearest molecular marker suggesting their possible use in marker-assisted breeding for groundnut in Zambia.

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Biological and genetic basis of peanut nodulation: More questions than answers

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Peanut rhizobia enter the peanut root primarily through a crack at the sites of lateral root emergence and spread intercellularly in root cortical cells, which does not resemble the infection processes in other model legume species. Understanding the biological and molecular mechanisms of peanut nodulation will reveal novel insights into nodule organogenesis. To study the biology of peanut nodulation, single colonies from peanut nodules were sampled for 16S rDNA sequencing. Seven different rhizobia strains belonging to two different genera, Bradyrhizobium and Paenibacillus were identified. All of the strains are Gram-staining positive, and can infect different peanut types and form functional nodules but with varied nodule number and nitrogen fixation rate. Two pairs of peanut non-nodulating and nodulating sister lines were infected by a single rhizobial strain. Nodulating sister lines produced nodules at nine days after inoculation while the non-nodulating lines didn't. To map the genes controlling peanut nodulation, the two non-nodulating lines were cross-pollinated with their nodulating sister lines to generate two F_2 segregating populations, respectively. The segregating ratio of nodulating:non-nodulating of the two populations were 9:7 and 3:1, respectively. Genotyping of the two pairs of sister lines revealed their genome identity of 93.6% and 84.6%, respectively. Two quantitative trait loci (QTLs) on chromosome B07 can respectively explain 29% and 35% of phenotypic variance of the two F_2 populations. Dissecting the biological and genetic basis of peanut nodulation and symbiosis through crack entry infection is critical for sustainable agriculture, specifically for developing crop cultivars with highly efficient symbiosis.

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Botryosphaeria rhodina as a biotic elicitor to enhance resveratrol biosynthesis during peanut seed germination

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Resveratrol is a major phytoalexin produced by plants in response to various stresses and promotes disease resistance. In our previous experiments, a fungal contaminant has been frequently observed during peanut kernel germination, and the resveratrol contents of those infected peanut cotyledons were much higher than those of the normally germinated ones. The fungal isolate was first subjected to molecular identification as *Botryosphaeria rhodina* RCYU 30101. In this study, *B. rhodina* involved in enhancement of resveratrol biosynthesis of peanut kernels during germination was conducted. When peanut kernels were surface-disinfected, artificially inoculated with the mycelial suspension of *B. rhodina* RCYU 30101 and germinated at 20 and 30^{o}C for 96 h, resveratrol contents increased substantially after 24 h of colonization. The highest resveratrol levels for the *B. rhodina* colonized kernels were 316.5 and 354.9 μg/g freeze-dried kernel weight after 96 h of incubation at 20 and 30 ^{o}C, respectively. Similarly, a significant effect on enhancement of *trans*-piceid biosynthesis by artificial infection with *B. rhodina* was also observed, and the highest *trans*-piceid level was 104.7 μg/g after 96 h of incubation at 30 ^{o}C. For the un-inoculated kernels, the highest resveratrol and *trans*-piceid contents were only 31.0 and 31.5 μg/g, respectively. In conclusion, *B. rhodina* isolated from the germinating peanut kernels was demonstrated as a potent elicitor to enhance biosynthesis of *trans*-resveratrol and *trans*-piceid.

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POSTERS

Drought tolerance mechanisms for responses to pre and post flowering drought stress of groundnut in a dryland ecology

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Understanding the mechanisms of groundnut tolerance to pre and post flowering drought stress is important for improving its yield and phenological development in the drylands. The mechanisms of drought tolerance are known to be under variable genetic control. The aim of this study was to investigate the mechanism of drought tolerance of various groundnut genotypes to pre and post flowering drought stress. Screen house trials were undertaken between March and June 2014. Three moisture management treatments were imposed on 10 groundnut cultivars. Three cultivars (Ex-Dakar, Samnut 23 and Samnut 24) are known to have some drought tolerance characteristics; two cultivars (Samnut 21 and Samnut 22) do not tolerate drought, while the drought response of the remaining five cultivars (Samnut 25, Samnut 26, Sabiya, Kwankwaso and Yar Digir) is not known. The water managements were: Field Capacity throughout the period of experimentation (FCT), Imposition of Pre-Flowering Moisture Stress (PrFS) and Imposition of Post Flowering Stress (PoFS). Stomatal conductance and relative water content (RWC) were recorded at 10, 15, 20, 25, 30, 35, 40 and 45 days after emergence. Total dry matter samples (shoots and pods) were collected at 15 and 25 days after emergence, R5 and R7. From these samples, shoot and pod growth rates were calculated. Two of the drought tolerant cultivars were found to tolerate only pre-flowering drought and only Ex-Dakar tolerate both pre and post flowering drought stress. Among the two susceptible cultivars, Samnut 22 was found to tolerate post-flowering stress, but was susceptible to pre flowering stress. Samnut 26 and Kwankwaso tolerated both pre and post flowering stress, while Sabiya and Yar Digir were susceptible to both stresses. Most of the cultivars adopted the mechanism of conserving water by reducing transpiration to maintain high RWC. Only Samnut 24 showed the mechanism of improving assimilate partitioning to the pods at grain filling phase. The knowledge gathered could be used for breeding groundnut that will be suited to the drylands in order to escape periods of intermittent drought.

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Impact of groundnut rosette disease on nutritive value and elemental composition of four varieties of peanut (*Arachis hypogaea*)

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Proximate and elemental composition of four peanut genotypes graft-inoculated with groundnut rosette disease (GRD) was examined. All genotypes succumbed to the disease, expressed typical GRD symptoms and tested positive from *Groundnut rosette assistor virus* infection by triple antibody enzyme-linked immunosorbent assay (TAS-ELISA). Generally, moisture content decreased in seeds of virus-infected plants compared to the healthy controls. Protein content was significantly increased in genotypes Otuhia and Nkosuor. With the exception of genotype Sinka, the differences between the ash content of seeds from virus-infected and healthy plants were generally non-significant. Carbohydrate content increased significantly in seeds of virus-infected plants of Nkosuor and Sinka but decreased in

that of Otuhia and Yenyawoso. The fat and energy content increased significantly in the virus-infected nuts of all the genotypes except Nkosuor. Instrumental neutron activation analysis measured 10 elements (Mn, Al, Na, Ca, K, Mg, V, Cl, Fe and Zn) in leaves, stems and seeds of both infected and healthy plants. GRD infection significantly increased Al, Cl and K content in all plant parts, irrespective of genotype. Na content decreased significantly in infected stems but increased in seeds of infected plants. The concentration of Mg did not significantly differ within seeds but increased significantly in stems of virus-infected plants. V content was significantly higher in leaves and stems of infected plants. Mn, Fe, Ca and Zn did not show any consistent change in content with respect to genotype and plant part. The work presented here represents the first detailed report on the effect of GRD on the quality of peanuts.

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Mapping of differentially expressed genes of *Arachis stenosperma* under *Meloidogyne arenaria* infection onto *Arachis duranensis* pseudomolecules

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Root-knot nematode, *Meloidogyne arenaria*, is one of the most important plant-parasitic nematodes because of their wide host range and widespread distribution. World-wide, it causes severe economic damage to the peanut crop. The wild species *Arachis stenosperma* has been identified as possessing near immunity to this pest. RNA-seq was conducted using plants of *A. stenosperma* inoculated with the nematode *M. arenaria* race 1 after 3, 6 and 9 days and the control assay. 30,838,392 paired-end reads were obtained after filtering and adaptor trimming, which were mapped to the *A. duranensis* V 14167 transcriptome assembly, and the further differential expression analyses were done using the TopHat and Cufflinks pipeline. From all of the conditions together, we recovered 58 up-regulated genes (FPKM >100 and P-value < 0.01) and 34 down-regulated putative genes (FPKM <100 and P-value < 0.01). A proportion of these differentially expressed were located in the vicinity of QTLs (chr 2 and 9) previously associated to the resistance response to this nematode. We found, after comparing against a protein database, a putative disease resistance protein (TIR-NBS-LRR class) in the linkage group 2, which seems to be up-regulated under nematode inoculation. This analysis allowed us to identify interesting genes which may play an important role in resistance to root-knot nematode attack and can be used as markers for foreground selection in breeding programs aiming the introgression of resistance to RKN from wild species.

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Analysis of mutant populations for association of taxonomic and productivity traits with transposable element (TE) markers in peanut

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Mutations have played a significant role in the evolution of subspecies and botanical varieties in peanut. These mutations largely involved the activity of transposable elements, which are widely distributed in the peanut genome and have transpositional preference to genic regions. Hence, the recently developed

TE marker system, which can detect differential transpositional activity between individuals, when used among a large number of mutants of independent origin but differing for taxonomic traits and productivity traits would not only allow associating specific genetic changes with the phenotypic changes, but also permit validating such associations by comparing mutants and their parents. Forty two mutants (Developed at UAS, Dharwad, India) displaying shifts in taxonomic traits, and thirty eight mutants (Developed at BARC, Mumbai, India) exhibiting significant shifts in productivity traits, foliar disease resistance and quality traits were evaluated during the rainy season (July-October) of 2012 and 2013. Mutants along with their parents were also genotyped with 60 transposon-specific markers. Single marker analysis, AMOVA, locus by locus AMOVA and Kruskal-Wallis ANOVA revealed the suitability of the two mutant populations for identifying the marker-trait associations. Significant association of AhTE205 with the main stem flowering was observed, where it could differentiate ssp. *hypogaea* from ssp. *fastigiata*. A few markers showing strong association with traits like number of pods/plant, pod yield/plant and shelling percentage, test weight, late leaf spot resistance, oil content, oleate content and O:L ratio were also identified and validated. These markers are also being validated using other populations before they are considered for peanut improvement.

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Identification of SNPs for *Arachis hypogaea* L. genotypes using GBS, RNA-Seq and WGS based on the two diploid reference genomes

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Peanut/groundnut (*Arachis hypogaea* L.) is one of the most important legumes in the world, especially due to high oil and protein content. Thus, it is an allotetraploid specie originated from a cross between two different diploids *A. duranensis* and *A. ipaensis* with A and B genomes respectively, followed by whole genome duplication. Due to this allopolyploidy, it has been more challenging to find allelic SNPs and polymorphic markers. Therefore, starting in 2008 in the USDA-ARS/UGA peanut breeding program on the Tifton Campus, 16 mapping populations were developed with different trait combinations such as disease resistance, drought tolerance, and pod morphologies. Thus, it has been imperative the development of strategies using high-throughput technologies to identify great amount of markers for genotyping such as breeding populations at a big scale. Thereby, some of the parents of the populations such as Tifrunner, NC3033, Florida 07, C76-16, SPT06-06, and New Mexico Valencia were taken as a reference in this study and high throughput technologies such as GBS (Genotyping by Sequencing), RNA-Seq, and WGS (Whole Genome Sequencing) libraries were developed as a source for SNP calling using known software. Thus, a comparison of these three technologies to call SNPs was made to obtain enough polymorphic markers for genotyping either from genic and genomic sources using two already known software, GATK and SAMtools, to confirm the allelic variations.

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A study on influencing factors of seed dormancy in peanut (*Arachis hypogaea* L.)

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Seed dormancy is an important agronomic trait which can affect planting, germination and harvesting in agricultural production. There can be some problems when too low seed dormancy levels lead to

germination before harvesting peanut (*Arachis hypogaea* L.). In this study, seeds from 16 cultivars were applied in a seed dormancy test. The results showed that the germination rate of dry seeds from 16 cultivars ranged from 0% to 100%. The germination frequencies of seeds from Huayu25, Huayu28 and Xuhua13 were 100%, while the germination frequencies of fresh seeds and drying seeds from the strong dormancy variety Huayu52 were 0%. Seed dormancy was closely related to maturity and seed moisture content. The change of endogenous hormones content in seeds showed that a lower ratio of GA/ABA and high levels of ABA at imbibition were related to the dormancy of peanut seed. There was no significant difference in dry seed ABA content of dormant variety Huayu52 and non-dormant variety Huayu28. In contrast to Huayu52, non-dormant variety Huayu28 exhibited a higher content in GA, IAA, 6-BA and a higher GA/ABA ratio.

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Expression analysis of cDNAs fragment encoding ABA 8'-hydroxylase in peanut (*Arachis hypogaea* L.) dormant seeds

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Dormancy is an adaptive trait that enables seed germination to coincide with favorable environmental conditions. Abscisic acid (ABA) is a positive regulator of dormancy induction and maintenance, while it is a negative regulator of germination. The ABA content of seeds is controlled both its synthesis and its deactivation. The major ABA catabolic pathway is triggered by ABA 8'-hydroxylation catalyzed by ABA 8'-hydroxylase, the cytochrome P450 CYP707A family. In this study, the change of endogenous hormone content in seeds showed that lower ratio of GA/ABA and high levels of ABA at imbibitions were related to the dormancy of peanut seed. A 5'-end gene fragment of ABA-8'-hydroxylase (*CYP707A*) was screened from the peanut dormant seeds and breaking dormancy seed by RNA-Seq. The fragment length of the ABA-8'-hydroxylase was 1672bp, and encoding 442 amino acids. Sequence alignment showed that it contained conserved cytochrome P450 domains, and revealed 98% similarity with the peanut *AhCYP707A1*（CDJ80018.1). Expression analysis showed that *AhCYP707A1 was* expressed ubiquitously in peanut roots, stems, leaves, flowers, gynophores and seeds, whereas *AhCYP707A1* mRNA predominantly accumulated in roots. The expression of *AhCYP707A1* was significantly up-regulated by 100mg/L ethephon to dormant seeds and in imbibed nondormant seeds, whereas it was only slightly increased in imbibed dormant seeds. This suggests that the expression of *AhCYP707A1* is correlated to the seed dormancy release.

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Transcriptome comparison of resistant and susceptible peanut (*Arachis hypogaea* L.) in response to *Ralstonia solanacearum*

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Bacterial wilt caused by *Ralstonia solanacearum* is a serious soil-borne disease of peanut (*Arachis hypogaea* L). The molecular basis of peanut in response to *R. solanacearum* remains unknown. The RNA-seq was employed to profile the transcriptome in the roots of peanut resistant (R) and susceptible (S) genotypes after *R. solanacearum* infection. A total of 4.95 x 10^8 raw sequence reads were generated

from 14 cDNA libraries and subsequently assembled into 279,454 unigenes. Two genotypes shared similar transcriptome pattern at early time points, and samples of R24 and S48 showed transcriptional similarity. The pairwise transcriptome comparison of time course was conducted at 6, 12, 24, 48 and 72 hours post inoculation with *R. solanacearumin* for a) between inoculated and control samples of each genotype, and b) between inoculated samples of R and S genotypes. A total of 54,622 differentially expressed genes (DEGs) were identified in both genotypes' responses to *R. solanacearum*, and significant differences of transcriptional profiles were observed between R and S genotypes. Down regulation of DEGs was dominated in both genotypes. The primary metabolisms were inhibited in both genotypes and stronger inhibition was observed in R genotype post inoculation. The defense related genes generally showed a genotype-specific down regulation in both genotypes. This study provides an overview of gene expression in peanut roots in response to *R. solanacearum* infection, and the candidate genes would be valuable for further study of molecular mechanisms of resistance to *R. solanacearum*.

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Cloning and functional analysis of fatty acid desaturase genes from peanut (*Arachis hypogaea* L.)

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Fatty acid desaturases are responsible for the insertion of double bonds into pre-formed fatty acid chains in reactions that require oxygen and reducing equivalents. In this study, 17 fatty acid desaturase genes were isolated from peanut, which belong to nine types. Quantitative real-time RT-PCR analysis indicated that the *AhFAB2-1*, *AhFAB2-2* and *AhFAD3-1* transcripts were more abundant in seeds, whereas the transcript abundances of *AhFAD7-2*, *AhSLD1-2* and *AhSLD1-3* were higher in stems than in the other tissues examined. The transcripts of *AhFAD2-2*, *AhFAD5*, *AhFAD6*, *AhFAD7-1* and *AhSLD1-1* were more abundant in leaves, whereas the transcript abundances of other six genes were higher in flowers. In addition, these genes showed different expression patterns during seed development. Expression analysis of 17 genes under conditions of cold, salt, drought and ABA stresses indicated that levels of *AhFAB2-2*, *AhFAD3-2*, *AhFAD4* and *AhFAD7-2* transcripts were distinctly enhanced after exposure to stress treatments. Heterologous expression in yeast was used to confirm the regioselectivity and the function of *AhFAD2-2* and *AhFAD6*. Linoleic acid, normally not present in wild-type yeast cells, was detected in transformants of these two genes. In addition, the activity of the *AhFAD6* gene was further confirmed by heterologous expression in the *Synechococcus* sp. PCC7942. Linoleic acid, normally not present in wild-type cyanobacteria, was detected in transformants of *AhFAD6* gene. The present study provides significant information to use in modifying oil quality and improving abiotic stress resistance of peanut through molecular breeding.

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Transcript-based SNP map and QTL analysis of plant architecture and seed traits of F_2 lines developed from an interspecific cross of *Arachis duranensis* x *Arachis cardenasii*

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Single nucleotide polymorphisms (SNPs) are used routinely in many species for genetic mapping and marker-assisted breeding because SNPs can be assayed rapidly and at a reasonable cost. In this study, we constructed an A-genome linkage map from SNPs derived from transcriptome sequencing of wild peanut species. The map is based on an F_2 population of 91 individuals obtained from the cross between the diploid *Arachis duranensis* (KSSc 38901) and *A. cardenasii* (GKP 10017). The former species is the most probable A- genome donor species to cultivated peanut, and the latter parent is highly resistant to several important diseases. SNPs were genotyped using KASP chemistry on either a Roche Light Cycler 480 or a Fluidigm Biomark HD, and using SNPType chemistry on the Fluidigm Biomark HD. This allowed for high throughput analysis (96 samples x 96 SNPs) in a single experiment for developing a genetic map or QTL analysis, and a more flexible design for marker assisted selection in a breeding lab. The map has 10 linkage groups, with 144 loci spanning a distance of 1,040.3 cM. This A-genome map was compared to the draft A-genome *A. duranensis* sequence using mapped markers, revealing a high degree of synteny between genetic and physical maps. QTLs were identified by composite interval mapping for plant architecture traits including leaf measurements, main stem height, and presence of flowers on the main stem, and for seed weight. The QTLs detected provide new information on plant architecture and seed traits important for domestication.

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A transcriptome map for geocarpic fruit development in *Arachis hypogaea*

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Peanut (*Arachis hypogaea*) is one of the few plant species that exhibit geocarpic fruit development; flowering and fertilization above ground and the ripening of the fruit below ground. This fascinating botany allows for the investigation of new gene interaction networks. As a portion of the Peanut Genome Initiative, we have sequenced the transcriptome of the reference genotype Tifrunner at different time points of various vegetative tissues along with a detailed series of flower, peg, and pod development. We used these sequences to assemble a reference transcriptome that is subgenome-specific using a genome-guided method and the diploid progenitor reference genome pseudomolecules. We identified a set of 827 transcripts that are specifically expressed during geocarpic development, including 395 B-genome derived and 432 A-genome derived. Of those, we could identify 25 putative homeologous pairs. Clustering of these genes using Self-Organizing Maps (SOM) revealed six co-regulated expression modules during geocarpic development. Annotation of the geocarpic specific genes revealed 10 auxin-related genes, including a putative ortholog of the auxin biosynthetic enzyme YUCCA, an auxin transporter, auxin response factors, ARF6 and IAA8, and a putative ortholog of the auxin efflux regulator PINOID. Additionally, a putative ortholog of FERONIA, which has been proposed to regulate growth in response to mechanical stimuli, is specifically expressed at pod swelling and during early pod development. Overall, these data represent a transcriptome map of the genetic regulation of geocarpic development in peanut.

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Phenotyping the RIL population of Tifrunner × C76-16 for drought tolerance in peanuts

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Increasingly warmer and drier conditions in the southeastern United States pose problems for peanut production, especially where irrigation is not available. 'C76-16', an advanced breeding line, has been identified as a unique drought tolerant source for peanut breeding programs. The RIL population derived from the cross of 'Tifrunner' × 'C76-16' should be useful in identifying QTLs underlying drought tolerance in peanut. The 156 RILs were planted in a plot of 76 cm length and 76 cm row spacing in environmental controlled rainout shelters at Dawson, GA using an augmented design in 2013 and 2014. The drought treatment begun at 60 DAP (days after planting). Various measures were used to estimate response to water deficit, including relative water content (RWC), specific leaf area (SLA), leaf density moisture content (LDMC), visual evaluation, and yield under drought stress. The results indicated the population segregated for the measured traits especially for yield under drought stress. A number of transgressive lines with enhanced drought tolerance, compared to 'C76-16', have been identified. Polymorphic markers between parents from 200 SSR candidate markers were identified and will be used for QTL identification. The feasibility of infrared images technology using in drought tolerance in peanut was explored in 2014 as a potential selection tool.

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PeanutBase: A community resource to help improve peanut varieties by integrating genetic, genomic, and trait information.

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PeanutBase (http://peanutbase.org) provides a web portal for genomics, genetics and trait information for peanut. The goal is to help peanut researchers improve peanut varieties by integrating genetic, genomic, and trait information. Genomic data (genome sequence, predicted genes, gene expression, etc.) is stitched together with breeder-centered information such as candidate genes, genetic markers, QTLs, trait information about breeding lines, and allele states for important markers. The genomes of the two wild progenitors of cultivated peanut, *A. duranensis* and *A. ipaensis*, have been sequenced and are available from PeanutBase – both as chromosomal sequence files, and in genome browsers. The browsers can be used to search and view genes and markers from peanut (cultivated and wild), and the corresponding genes from other species such as soybean and common bean. Many genetic traits have also been collected from various QTL studies and the data sets are now available from PeanutBase, and are viewable on interactive maps. PeanutBase was started in April 2013 at Iowa State University with funding from The Peanut Foundation and in-kind contributions from USDA-ARS.

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The potential role of oxidative stress in *Aspergillus flavus* survivability and aflatoxoin biosynthesis

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Aflatoxin contamination of food and feed occurs due to growth of *Aspergillus flavus*. This poses a serious health risk because of aflatoxin's toxic and carcinogenic properties which negatively impact human and livestock health. Colonization and subsequent aflatoxin production by *A. flavus* is typically associated with stressed crops in the field. The purpose of aflatoxin biosynthesis, however, is not well understood.

The objective of this study was to examine the possible association of oxidative stress, survivability and aflatoxin biosynthesis in different *A. flavus* isolates. Eight isolates were used, including both toxigenic (+) and atoxigenic (-) isolates: NRRL3357(+), A9(+), AF13(+), A1(-), K49(-), K54 (-), AF36(-), and Aflaguard(AF-). Fungal biomass was recorded following culture in yeast extract-sucrose (YES) media supplemented with hydrogen peroxide (H_2O_2) with a concentration gradient from 0 to 40 mM in 5mM increments for 7 days at 32°C in either stationary or shaking (100 rpm) conditions. Fungal growth was significantly affected at varying H_2O_2 concentrations, and the presence or absence of shaking did not significantly affect survivability. The toxigenic isolates were able to survive up to 20 to 35mM H_2O_2, and the atoxigenic isolates were able to survive up to 15 to 30mM H_2O_2. It was interesting that NRRL3357(+) survived to 20mM while K49(-) survived to 30mM. Overall, toxigenic isolates were able to survive significantly better than atoxigenic isolates over the H_2O_2 gradients. The general trend was more aflatoxin production with increased H_2O_2 concentration. Further investigation will be conducted to study the specific stress response signal genes and aflatoxin biosynthesis in different *A. flavus* isolates.

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Selection for high oleate phenotype with foliar disease resistance from backcross population in groundnut (*Arachis hypogaea* L.)

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Groundnut (*Arachis hypogaea* L.) is a rich source of edible oil in which oleic acid and linoleic acids are in major quantity (80%), the ratio of which determines the oil quality. In order to recombine high oleate trait with disease resistance, backcrossing (first and second) was done with recurrent parent (GPBD 4). In the present investigation, single and double backcrosses to the recurrent parent (GPBD 4) followed by individual plant selection was practiced from BC_1F_2 to BC_1F_5 and BC_2F_2 to BC_2F_3 generations with emphasis for high oleic acid content. There was significant improvement in oleic acid and O/L ratio from 50.55% to 69.18% and 1.65% to 7.89%, respectively, with reduction in linoleic acid level from 31.69% to 9.68% from BC_1F_2 to BC_1F_5 with single backcross, whereas, from BC_2F_2 to BC_2F_3 in two backcross generations, oleic acid and O/L ratio improved from 56.4% to 66.4% and 2.96% to 5.85% with reduction in linoleic acid from to 20.65% to 12.24% which could be further improved in advanced generations. O/L ratio and linoleic acid showed high heritability coupled with high genetic advance as percentage of the mean in all generations from BC_1F_2 to BC_1F_5 and BC_2F_2 to BC_2F_3 generations. There was considerable change from positive skewness to negative skewness observed for oleic acid and O/L ratio from BC_1F_2 to BC_1F_5 and BC_2F_2 to BC_2F_3 generation indicating that selection based on these traits could be effective in identifying superior lines with high oleate and foliar disease resistance.

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Molecular cloning and functional analysis of the Annexin gene family in peanut (*Arachis hypogaea* L.)

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Annexin, Ca^{2+} or phospholipid binding proteins, with many family members are distributed throughout many tissues during plant growth and development. Annexins participate in a number of physiological processes, such as exocytosis, cell elongation, nodule formation in legumes, maturation and stress response. In the present study, six different cDNAs and two partial cDNAs of peanut, *Arachis hypogaea* L. (*AnnAh1*, *AnnAh2*, *AnnAh3*, *AnnAh4*, *AnnAh5*, *AnnAh7*, *AnnAh6* and *AnnAh8*) encoding annexin proteins were isolated and characterized using a RT-PCR/RACE-PCR based strategy. The predicted

molecular masses of these annexins were 36.0 kDa with acidic pIs of 7.1, 6.15, 6.11, 8.58, 8.67 and 6.47 respectively. At the amino acid level, AnnAh1, AnnAh2, AnnAh3, AnnAh4, AnnAh5, AnnAh7 shared sequence similarity between 35.76 to 66.35% with each other. Phylogenetic analysis revealed their evolutionary relationship with corresponding orthologous sequences in soybean and deduced proteins in various plant species. Real-time quantitative assays indicated that these genes were differentially expressed in various tissues. Gene-expression analysis under stress conditions showed these genes regulation by drought, salinity, heavy metal stress, signaling molecules and low temperature. These results indicated that members of *AnnAh* gene family may play important roles in adaptation of plants to various environmental stresses.

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Cloning and expression analysis of four DELLA genes in peanut

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Peanut is one of the most important grain legumes cultivated in the world. It is a relatively drought-tolerant crop; however, the molecular mechanisms of its stress resistance are poorly understood. DELLA proteins, negative regulators of gibberellin signaling pathway in plants, promote survival of plants in adverse environments. In this study, four *DELLA* homologue genes were isolated using peanut transcriptome sequences. Molecular phylogenetic analysis revealed that these four AhDELLAs fall into three distinct groups: AhDELLA1 and AhDELLA2 clustered into two distinct groups, while AhDELLA3 and AhDELLA4 were in one group, which was separated from other DELLAs. qRT-PCR results showed that these four *DELLA* genes expressed differentially in various peanut tissues. The *AhDELLA1* and *AhDELLA2* genes were expressed ubiquitously in different tissues. *AhDELLA3* and *AhDELLA4* showed much higher expression level in flowers and seeds as compared with other tissues. The expression of four *AhDELLA* genes was temporally induced by PEG 6000 treatment. The *AhDELLA3* and *AhDELLA4* transcripts were significantly induced by NaCl treatment, while the expression of *AhDELLA1* and *AhDELLA2* did not change significantly under salt stress. The possible role of DELLA proteins in peanut development and responses to abiotic stresses is discussed.

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Three high oleic groundnut varieties from SPRI

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Huayu 51, Huayu 52 and Huayu951 were developed by Shandong Peanut Research Institute (SPRI) and released by the Anhui Provincial Crops Approval Committee. Huayu51 and Huayu951 were derived from a cross between Xuhua13 and P76 following modified pedigree method. Huayu52 was derived from a cross between Qinglan2 and P76 following modified pedigree method. Huayu51 and Huayu52 are small-seeded groundnut varieties; Huayu951 is a large-seeded groundnut variety. The three varieties have an erect growth habit, sequential flowering. It's mature in 130 days in the spring season. Huayu52 shelling outturn is 76.63% and the 100-pod mass is 190.00g and 100-seed mass is 76.29g. Its seeds contain 81.45% oleic content. The oleic acid/linoleic acid (O/L) ratio of Huayu52 was 26.97. Huayu51 shelling outturn is 74.18% and the 100-pod mass is 173.75g and 100-seed mass is 64.45g. Its seeds contain 80.31% oleic content. The oleic acid/linoleic acid (O/L) ratio of Huayu51 was 23.92。Huayu951 shelling

outturn is 70.3% and the 100-pod mass is 201.8g and 100-seed mass is 84.1g. Its seeds contain 80.47% oleic content. The oleic acid/linoleic acid (O/L) ratio of Huayu951 was 27.80.

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Genetic variability and marker detection for rust resistance in recombinant inbred lines and backcross inbred lines of groundnut (*Arachis hypogaea* L.)

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A F_6 mapping population and backcross populations (BC_1F_4 and BC_2F_3 BC_3F_2) were developed from the cross between the susceptible parent GPBD-5 and resistant parents ICGV 86699 and ICGV 99005 to dissect the genetic variation and SSR markers linked to the rust resistance in groundnut. Genetic variability revealed that there were highly significant differences among recombinants for rust reaction. Less differences were observed between PCV and GCV for rust reaction in both crosses, which indicated a greater role of genetic components. High values of heritability (>80%) genetic advance and genetic advance as percent mean was observed for rust reaction in F_6 and backcross populations. Bulk segregant analysis in the segregating populations of both crosses (GPBD-5 × ICGV 86699 and GPBD-5 × ICGV 99005) indicated Tc4g10 marker was putatively linked to the rust resistant gene. The association of the putative marker identified based on DNA pooling from the selected segregants was established by single marker analysis (SMA). In the F_6 population of both crosses GPBD-5 × ICGV 86699 and GPBD-5 × ICGV 99005, the Tc4g10 marker accounted for 72.40% and 50.60% total variation, respectively. Tc4g10 marker accounted for 67.10%, 38.40% and 61.30% total variation in the cross GPBD-5 × ICGV 86699, and the same marker accounted for 73.8%, 54.7% and 84.4% total variation in the cross GPBD-5 × ICGV 99005 in BC_1F_4, BC_2F_3 and BC_3F_2, respectively. This marker can be used in marker assisted selection for rust resistance in groundnut improvement programs.

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Backcross breeding in groundnut (*Arachis hypogaea* L.)

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Rust caused by *Puccinia arachidis* Speg. is the most serious disease of groundnut and causes substantial yield loss and reduces the fodder and seed quality. Recombinant inbred lines (F_6) were generated by SSD method from cross GPBD-5 × GPBD-4 and the rust resistant plants were backcrossed to the recurrent parent (GPBD-5) to develop a backcross population (BC_1F_4). The objective of this experiment was to study the possibilities of linkage break-down between yield component traits and rust resistance in groundnut. Analysis of data revealed that there was a shift in correlation from negative (F_6) to positive significant direction (BC_1F_4) between pod yield per plant with plant height and between plant height with number of primary branches. Similarly there was linkage break-down between negative significant to positive significant association of number of primary branches with number of pods per plant, kernel yield per plant and shelling percent at both genotypic and phenotypic levels. Altogether a desirable shift in association and the proof of broken repulsion phase linkage and release of concealed variability, which is useful in plant breeding, provides a lot of scope for selection. Unchanged negative association between pod yield per plant with reaction to rust and shelling percent in both phenotypic and genotypic level in both the populations, indicated the operation of strong linkage blocks and which requires an intensive selection to combine disease resistance with yield. Otherwise inter-mating of highly extreme segregants in the populations also would cause breakage of these stubborn linkages. Thus, for yield component traits, backcrossing of selected plants is more rewarding than the single cross and advance by single seed decent method of breeding in groundnut.

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Association of relative water content (RWC) and specific leaf weight (SLW) as indicators of drought tolerance in peanut (*Arachis hypogaea* L.)

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A total of 110 advanced breeding peanut lines received from ICRISAT, Hyderabad along with local checks were evaluated in vertisol field at Main Agricultural Research Station of University of Agricultural Sciences, Dharwad, Karnataka, India during the 2011 post rainy season under normal watered (field capacity) and post-flowering (40-75 days) moisture stress conditions. The comparison of the superior genotypes with least reduction (<10%) in pod yield with those genotypes with higher (>25%) reduction in pod yield resulted in identification of relative water content (RWC) and specific leaf weight (SLW) as the critical physiological traits that are not reduced in tolerant genotypes compared to large differences recorded in susceptible genotypes. The mean performance of superior 10 genotypes was 2.85 t ha^{-1} in normal watered condition and 2.63 t ha^{-1} under moisture stress conditions leading mean pod yield reduction of 7.6%, whereas, susceptible genotypes (8) recorded mean pod yield of 2.52 t ha^{-1} under normal and 1.79 t ha^{-1} under moisture stress conditions, thus resulting in 29.1% reduction in pod yield. However, RWC was maintained at higher level both under normal (74.2%) and moisture stress (74.6%) conditions in tolerant superior genotypes, whereas, in susceptible genotypes, there was significant reduction in RWC from normal (72.1%) to water stress (59.8%) conditions at 75 days after sowing. In susceptible genotypes, there was significant reduction in specific leaf weight both at 45 (22.7 to 16.8g/cm^2) and 75 (20.5 to 15.5g/cm^2) days after sowing. The superior performance of these identified tolerant genotypes was confirmed by their field testing in drought prone locations, Bijapur and Hanumanmatti, thus proving the value of RWC and SLW as strong indicators of drought tolerance in peanut.

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Quantitative trait locus analysis and construction of consensus genetic map for agronomic traits based on three F_2 populations

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Cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid (AABB, 2*n* = 4x = 40), valued for its edible oil and protein. Linkage mapping based on bi-parental segregating populations is a popular and successful method to explore genetic architecture of important traits. The objective of this study was to develop an integrated linkage genetic map with three F_2 mapping populations, namely Zhonghua 10 × ICG12625 (ZI), Fuchuandahuasheng × ICG6375 (FI) and Xuhua 13 × Zhonghua 6 (XZ) and to apply them in mapping QTLs for agronomic traits. Linkage analysis of SSR markers resulted in the mapping of 1877.3 cM with 470 loci, 1675.3 cM with 347 loci and 1337.7 cM with 228 loci in the ZI, FI and XZ population respectively. Using 79 markers common to the three maps, an integrated map with 792 SSR loci and total map distance of 2068.9 cM was constructed. All the loci were mapped to 20 linkage groups (LG) and assigned to A1-A10 for A genome and B1-B10 for B genome, respectively. A high level of phenotypic variation, broad-sense heritability and significant correlations were observed in the three $F_{2:3}$ populations for agronomic traits. For agronomic traits, we identified 24 QTLs with 1.69 to 18.70% of explained phenotype variance in ZI population, 26 QTLs with 2.09 to 15.03% of explained phenotype variance in F_1 population and 16 QTLs with 1.25 to 16.73% of explained phenotype variance in XZ population. Several QTLs for multiple traits were overlapped with each other, reflecting the phenotypic correlation between these traits. The majority of QTLs exhibited obviously dominance or over-dominance effect on agronomic traits, indicating heterosis may be critically important in determining important traits of cultivated peanut. A linkage genetic map of high-density SSR markers could be hopeful in exploiting genomic duplication and arrangement and would be beneficial to assist in assembly of scaffolds in genome sequencing of *Arachis hypogaea*.

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Marker assisted backcrossing (MABC) to improve oil quality in peanut

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Peanut kernels contain 48-50% oil, and Oleic (O) and Linoleic (L) fatty acids constitute about 80% of the oil. The O/L ratio in normal peanut is about 2, however, in case of FAD mutants it can go as high as >20. High O/L ratio has benefits to consumer's health as well as food processing industry. MABC program was initiated to enhance O/L ratio of six elite genotypes that have >50% of seed oil content. Allele specific markers and CAPS markers were validated and used for marker assisted selection of *FADA* and *FADB* mutants in backcross populations. Individual plants in BC_1F_1, BC_2F_1 and BC_3F_1 were genotyped and for FADA FADB mutant alleles and hybridity confirmed plants were advanced. Based on genotyping in BC_1F_2 and BC_2F_2, homozygoted for both mutant alleles were selected. Subsequently progenies in BC_1F_5 and BC_2F_4 generations were phenotyped for O/L ratio and progenies with recurrent parent phenotype and O/L ratio varying from 8 to >20 were selected. The best performing selections will be advanced to preliminary yield trials during 2014/15 post-rainy season. Fatty acid contents were estimated using near infrared reflectance spectroscopy (NIRS) and further confirmation of high Oleic acid content was done using Gas Chromatography (GC). Lines homozygous for both *FADA* and *FADB* mutation had relatively higher O/L ratio than the lines that are homozygotes to one either of the alleles. Similarly form the

crosses, ICGV 06420 × SunOleic 95R and ICGV 05100× SunOleic 95R, progenies with O/L ratio >20 were selected and advanced.

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Analysis of differentially expressed leaf proteins and their interaction inrResponse to water stress in peanut

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Water stress predisposes peanut to pre-harvest aflatoxin contamination. Major changes during water stress include oxidative stress leading to destruction of photosynthetic apparatus and other macromolecules within cells. To gain better understanding of the effects on molecular and cellular functions, two peanut cultivars with diverse drought tolerance characteristics were subjected to water stress (WS). Leaf samples at different stress intervals were subjected to proteome analysis using two-dimensional electrophoresis complemented with MALDI/TOF mass spectrometry. Ninety-six proteins were differentially expressed in response to water stress in both cultivars. Three proteins (glutamine ammonia ligase, chitin class II and actin isoform B) were unique to tolerant cultivar. Four proteins (serine/threonine protein phosphate PP1, choline monooxygenase, peroxidase 43 and SNF1-related protein kinase regulatory subunit beta-2) which play a role as cryoprotectants through signal transduction and defense were induced in the drought tolerant cultivar following WS. Several of the leaf proteins that were over expressed in the tolerant cultivar to WS were suppressed in the susceptible cultivar. Protein interaction prediction analysis suggests that more proteins interacting in tolerant cultivar were shown to activate other proteins in directed system response networks. Interologs of these proteins were found in Arabidopsis and we believe that similar mechanism might exist in peanut.

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Development/identification of new genetic sources for high oleic acid in groundnut (*Arachis hypogaea* L.)

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Fatty acid composition of the oil determines its stability and nutritive value. High oleic acid and low linoleic acid make groundnut oil ideal for storage and better human health. Because of unavailability of any cultivar choices having high oleic acid in developing and underdeveloped countries, it makes it difficult to steadily develop oil quality improvements in groundnuts. To develop or identify the new genetic resource for improved oil quality, adapted cultivars, one with small seed size (GPBD-4) and another with bold seeds (TPG-41) coupled with slightly higher oleic acid (50-60%) were subjected to induced mutagenesis using chemical (EMS-0.5%) and physical mutagens (Gamma rays-200 and 300 Gy). Mutagen treatment with EMS (0.5%) in GPBD-4 and gamma rays at 300 Gy in TPG- 41 were effective in increasing the variability for fatty acid composition. Greater magnitude of induced variability was found for oleic acid (37.40%-75.16%), linoleic acid (9.01-40.30%) and O/L ratio (0.95-8.34). Mutant, GE-113 (74.48%, 9.17% and 8.12) and GM 4-3 (71%,16.18% and 4.39) recorded significant increase in oleic acid,

reduced linoleic acid and significantly increased O/L ratio compared to control GPBD-4 (50.87%, 29% and 1.76). The ICRISAT mini core collection consisting of 184 accessions was also screened to identify the available germplasm collection for high oleic acid. More than 50% of the accessions had higher oleic acid content compared to normal widely grown cultivars like TMV 2 (40%), among them ICG 2381 and ICG 6913 recorded high mean oleic acid (73.36 and 66.75%), low linoleic acid (10.61 and 11.29%) and better O/L ratio (6.91 and 5.90). These high oleic accessions and mutants were tested with *ahFAD* CAPS maker associated with high oleic acid and could find reported mutant allele of *FAD2A* but reported that the mutant allele of *FAD2B* could not be detected in any of these new genetic resources identified for high oleic acid content. These new genetic resources, some of them with foliar disease resistance, could serve as additional sources of high oleic acid in future groundnut breeding programs for oil quality improvement.

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QTL mapping and quantitative disease resistance to TSWV and leaf spots in a recombinant inbred line population SunOleic 97R and NC94022 of peanut (*Arachis hypogaea* L.)

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Peanuts are susceptible to a range of diseases, such as *Tomato spotted wilt virus* (TSWV), early leaf spot (ELS) and late leaf spot (LLS). Breeding line NC94022 has been identified with the highest resistance to TSWV in the field. Quantitative trait loci (QTL) mapping is a highly effective approach for studying genetically controlled disease resistance. A population of 352 recombinant inbred lines (RILs) from a cross between 'SunOleic 97R' and 'NC94022' was developed. A genetic map was constructed with 248 SSR marker loci across 21 linkage groups (LGs) and a total map length of 1425.91cM with a marker interval of 5.7 cM. QTL analysis using phenotypic data from multiple-locations and years (2009-2013) resulted in the identification of 89 QTLs on 11 LGs with phenotypic variance (PV) ranging from 3.46% to 30.15%, and 22 QTLs were found to be major QTLs (PV >10%), including 41 (10 major ones on 9 LGs), 35 (eight major ones on 6 LGs) and 13 (four major ones on 3 LGs) QTLs for resistances to ELS, LLS and TSWV, respectively. Consistent QTL regions expressed in more than two environments were also identified for all three diseases on LG1 (PV up to 15%), LG13 (PV up to 17%) and LG1 (PV up to 29%), respectively. It was interesting to note a "gene rich" LG for ELS and TSWV in LG1. Further fine-mapping of the QTLs will be needed in order to identify the potential resistance gene(s) and decipher the molecular basis of disease resistance mechanisms in peanuts.

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Development of introgression lines and advanced backcross QTL analysis for disease resistance, oil quality and yield component traits in peanut

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Ploidy difference between wild *Arachis* species and cultivated genotypes hinder transfer of useful alleles for agronomically important traits. To overcome this genetic barrier, several synthetics have been developed at ICRISAT. Furthermore, two synthetic amphidiploids viz., ISATGR 1212 (*A. duranensis* ICG 8123 × *A. ipaensis* ICG 8206) and ISATGR 265-5A (*A. kempff-mercadoi* ICG 8164 × *A. hoehnei* ICG 8190) have been used to generate two advanced backcross (AB) populations. The AB populations namely AB1 (ICGV 91114 × ISATGR 1212) and AB2 (ICGV 87846 × ISATGR 265-5A) have been genotyped with DArT and SSR markers. Based on these marker genotyping data, 258 (253 DArT and 5 SSR) and 1043 (1034 DArT and 9 SSR) loci have been mapped on 20 and 19 linkage groups covering a total map length of 1415.7 cM and 1500.8 cM with map density of 5.49 and 1.44 cM for AB1 and AB2 populations, respectively. These populations have also been phenotyped for disease resistance (late leaf spot, rust and peanut bud necrosis), oil quality (oleic and linoleic acid ratio, saturated and unsaturated fatty acids), and yield components (pod yield, 100 seed weight, sound mature kernels and shelling percentage). Quantitative trait locus (QTL) analysis using above mentioned genotyping and phenotyping identified 15 QTLs (PV 6.68-14.84%) in AB1 population while 34 QTLs (PV 6.9-67.8%) in AB2 population for disease resistance, oil quality and yield component traits. The AB-QTL approach facilitated simultaneous identification of QTLs and introgression of wild genomic regions associated with traits of interest into the cultivated gene pool of peanut.

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Identification of conjoint genomic regions for multiple traits using RIL populations through meta-QTL analysis in peanut

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In recent years, several quantitative trait locus (QTL) studies have been conducted for tolerance to drought, resistance to foliar diseases and yield-related traits in peanut. Number as well as position of the identified QTLs for a given trait, however, differed in different studies mainly because of the use of different mapping populations and different kind of markers. For instance, 205 QTLs with varied degree of phenotypic variance (PV) were identified for drought and related traits in three mapping populations (TAG 24 × ICGV 86031, ICGS 76 × CSMG 84-1 and ICGS 44 × ICGS 76). Therefore, it is really challenging to target the appropriate QTLs in molecular breeding for improving drought tolerance in peanut. Hence, there is need to conjoin the results of these studies to make a better conclusion on number and positions of QTLs for their use in genomic-assisted breeding (GAB). The present study has been planned on combining the QTL information detected in three different RIL populations into a consensus map and identifying important genomic regions through meta-QTL analysis. The meta-QTL analysis confined the earlier identified QTLs to 42 meta-QTLs. Mean confidence interval (CI) of 42 meta-QTLs was found 5.51 cM and mean phenotypic variance of the meta-QTLs was estimated as 7.34%. Interestingly, 16 meta-QTLs harbored more than two component traits. Efforts are underway to collate more QTLs from additional RIL populations. In summary, meta-QTL analysis has redefined the QTL positions irrespective of the genetic background and provided meta-QTLs with more confidence and higher precision for developing superior cultivars through molecular breeding.

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Functional identification of the oleosin gene promoter in peanut (*Arachis hypogaea* L.)

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The oleosin gene promoter sequence *OL1584* (EU518466) was isolated from genome DNA of peanut cultivar Fenghua 3 by the PCR technique according to the sequence of *oleosin17.8* gene (EF695400) published on GeneBank. Sequence alignment analyses revealed that *OL1584* and the promoter region of *oleosin17.8* shared 99.37% homology. *OL1584* contains TATA-Box,CAAT-Box, two AT-Rich elements, two RY elements, five CATG boxes, five E-Boxes, six GATA-Boxes, two TACACAT elements and 14 AAAG boxes. The expression vector pB-OL1584 was constructed by attaching *OL1584* into the plasmid pBI121 with CaMV35S promoter resected. *OL1584* fused with reporter gene encoding β-glucuronidase (GUS) in expression vector pB-OL1584. Two lines of T_5 generation of a transgenic Arabidopsis plant were obtained by transformed transformation with expression vector pB-OL1584 in *Agrobacterium tumefaciens* LBA4404. Meanwhile, two lines of T_4 generation transgenic plant were obtained by transformed transformation with expression vector pBI121 (CaMV35S promoter fused with GUS gene). By analyzing GUS activity of the transgenic Arabidopsis plant lines harboring *oleosin* promoter OL1584-GUS gene, we found that GUS activities have significant differences in different tissues of the same plant, and that is highest in root, which is followed by pod, stem and leaf. In addition, GUS activities also have a significant difference among the same organ in different transgenic plant lines. GUS activity is 4.01 nmol/mg(protein) /min in the root of transgenic plant O2-18(harboring OL1584-GUS gene), which has the highest enzymatic activity among all the plants; 2.60nmol/mg(protein)/min in the pod; and 0.73 nmol/mg(protein)/min in the leaf of O2-18. Detecting the relative expression level of GUS gene in the transcriptional level, we found which being the same tissue specificity in different plants as well as the enzymatic activity. These results suggest that the level of expression of peanut *oleosin* promoter *OL1584* in *Arabidopsis* is the highest in root, followed by the pod, and has little expression in stem and leaf. Comparing the GUS activity in transgenic Arabidopsis plants O2-18 and I1-89（harboring CaMV35S -GUS gene, we found that the GUS activity in O2-18 is 0.66 times of I1-89 in the root, 1.14 times in the stem, 1.04 times in the leaf and 2.03 times in the pod. This indicates that the expression the peanut *oleosin* promoter *OL1584* in Arabidopsis is higher than that of CaMV35S promoter in the pod, but lower in the root, and there is no significant difference in the stem and leaf.

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K 1341, a high yielding large seed Virginia bunch groundnut variety with multiple resistances for biotic and abiotic traits released for cultivation

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Large-seeded peanuts are likely to attract a premium price in the world market of edible peanuts. However, foliar diseases such as late leaf spot can cause significant yield loss and affect the seed quality. Application of fungicide to control foliar diseases causes differential changes in seed mass, total oil, protein content and fatty acid composition. Therefore, K 1341 a high yielding large seed bunch variety with multiple resistance for biotic and abiotic traits was developed with pedigree {ICGV86522XICG (FDRS) 10} XICGV91172. Modified bulk method of breeding was followed and K1341 was selected in the segregating material and evaluated during kharif trials over nine years. The mean pod and seed yield were 1523 and 1050 kg/ha, with an increase of 85.8% over check varieties over years. K1341 was evaluated in the AICRPG trials over seven locations for two years. The mean pod and seed yield were 2171 and 1580 kg/ha, with an increase of 37.0 % over large seeded check variety. Performance in the

field under front line demonstrations was extremely good with a mean yield of 5341kg/ha with an increase of 105% over the check. The quality parameters of K1341, namely hundred seed weight 70-75 g, protein content 26.9%, oil 48.6%, total soluble sugars 14.5% oleic acid 49.0% linoleic acid 29.3% and O/L ratio 1.67, showing high shelf life of the oil were extremely desirable. The Incidence of foliar diseases is also very low. The molecular studies of are in progress to better understand the drought tolerance and defense mechanisms.

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Comprehensive association analysis for 50 agronomic traits in peanut using the 'reference set' comprising 300 genotypes from 48 countries of the semi-arid tropics of the world

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Peanut is an important source of nutrition and supports livelihood for millions of small-holder farmers in the semi-arid tropics (SAT) of the world. Newly developed peanut cultivars could not yield to its original potential due to several biotic and abiotic stress factors. Under such circumstances, the integration of genomics tools with on-going genetic improvement approaches is expected to facilitate accelerated development of improved cultivars. In this context, high-resolution genotyping and multiple season phenotyping data for 50 important agronomic, disease and quality traits were generated on the 'reference set' of peanut. The above data were then subjected to comprehensive analyses for allelic diversity, population structure, linkage disequilibrium (LD) decay and marker-trait association (MTA) in the 'reference set'. Each genotype of this set can be differentiated with each other using either a unique allele detected by a single SSR or a combination of unique alleles by two or more than two SSRs. The DArT markers (2.0 alleles/locus, 0.125 PIC) showed lower allele frequency and polymorphic information content (PIC) than SSR markers (22.21 alleles /locus, 0.715 PIC). Multi-allelic SSRs identified three sub-groups while the LD simulation trend line based on squared-allele frequency correlations (r^2) predicted LD decay of 15-20 cM. Association analysis resulted in identification of 524 highly significant MTAs (p value >2.1x10-6) with wide phenotypic variance range (5.81-90.09%) for 36 traits. These MTAs upon validation may be deployed in genomics-assisted breeding for developing improved peanut cultivars with enhanced resistance/tolerance to different stresses and higher pod yield with improved oil/ seed/ nutritional quality.

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Molecular marker discovery and validation from peanut (*Arachis hypogaea* L.) transcript sequences

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The development of molecular markers in cultivated peanut has been growing rapidly. This study focused on discovering and validating molecular markers from two peanut transcript databases: a NCBI EST dataset with 253,274 sequences and an in-house 454 RNAseq dataset with 288,701 sequences. Both datasets were assembled independently into a unigene set for SSR and SNP discovery. In total, 6,455 novel SSRs with primer sequences were developed after removing the sequences potentially containing known SSRs. Out of the 6,455 SSRs, 380 representing various SSR types were selected for PCR validation. The amplification rate was 89.2%. Twenty-two (6.5%) SSRs were polymorphic between at least one pair of four genotypes: Tifrunner, NC3033, Georgia Green, and C7616. SSRs with a 'AT/TA' motif have the highest polymorphism. By aligning the reads to assembled contigs, 11,902 SNPs and 2,949 INDELs were detected. To validate the SNPs, Sanger sequencing of PCR products targeting 110 SNPs was conducted. Sequence comparisons of the PCR products from four different genotypes revealed 13 true SNPs between tetraploid genotypes and 193 homoeologous SNPs, which were the SNPs between A and B genomes within genotypes. The results of this study enrich the peanut molecular marker tool box by providing over 6000 novel SSR markers covering the whole peanut genome and by providing the credentials for true peanut SNP marker development.

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Evaluation of groundnut genotypes for resistance to aflatoxin contamination

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Groundnut (*Arachis hypogaea* L.) is one of the premier oilseed crops of the world with export potential. Aflatoxin contamination of groundnut caused by *Aspergillus flavus* is a widespread serious problem in most groundnut-producing countries where the crop is grown under rain-fed conditions which adversely affects health of consumers of groundnut and its products. The marketability of contaminated produce, particularly in international trade is diminished to nil due to stringent standards of permissible limits on aflatoxin contamination set by the importing countries. Alleviation of aflatoxin contamination through genetic manipulation has been attempted in many groundnut producing countries. Breeding resistant cultivars is possible only when there are sources with stable, high levels of resistance to different mechanisms are available. Therefore, efforts to identify resistant genotypes would make other measures more effective. In the present investigation 10 cultivars, 34 germplasm accessions and 25 advanced breeding lines were screened by following spore spray and pin prick method. In spore spray method, six genotypes showed moderately resistant reaction (ICG-1122, ICG-2857, ICG-3336, ICG-7633, ICG-14985 and ICGV-02266), twenty one showed susceptible reaction and 42 showed highly susceptible reaction. While in pin prick method, only ICGV-02266 showed moderate resistant reaction and the remaining genotypes (68) showed a highly susceptible reaction. ICGV-02266 recorded moderate degree of resistance in both the methods of screening and showed both seed coat and cotyledon resistance, indicating the scope for its utility in aflatoxin resistance breeding in groundnut.

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K 1501, a high yielding large seed Virginia bunch confectionary groundnut at agricultural research station, India

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K 1501, a high yielding large seed Virginia bunch confectionary peanut variety with multiple resistances

for biotic and abiotic traits was developed by crossing very popular commercial groundnut variety Kadiri 4 with ICGV930179/P2. The F_1 was advanced during *kharif* to F2, and selection (2X068-002) was made in F_2 generation, and K1501 was evaluated in the Station level kharif trials at Kadiri over seven years. The mean pod and seed yield were 2175 and 11576 kg/ha, with an increase of 60.7 and 84.7% over large seeded check ICGV86564. K1501 was evaluated in the AICRPG trials over seven locations for three years. The mean pod and kernel yield were 2201 and 1482 kg/ha. The incidence of rust (3.8) is against 4.0 (TPG 41), 4.5 (TKG 19A), ELS (3.5) against 4.1 (M 13), 4.9 (TKG 19A), LLS (5.1) against 5.2 (TPG 41), 5.4(Somanath), peanut stem necrosis disease (9.2) against 18.1 (TPG 41), 14.8 (Somanath), collar rot (8.1) against 18.9(BAU 13), 17.6(M 13), 8.4(Somanath), 10.3(TKG 19A), stem rot (11.7) against 16.1(BAU 13), 14.1 (TPG 41). The incidence of peanut stem necrosis disease ranged from 2.5% to 6.0% against 14.6% to 25.0% in the check over three years of screening. Intensity of early leaf spot and late leaf spot ranged from 3.0 to 5.0 (Intermediate reaction) and 2.0 to 3.0 (resistant reaction) respectively. While the leaf minor damage was ranged from 1.8 to 6.4% and defoliators 5.6 to 12.6% against check 9.2% and 14.8% respectively.

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Physiological response and yield of paclobutrazol treated peanut (*Arachis hypogaea* L.)

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Experiments were conducted during two consecutive *kharif* seasons of 2013 and 2014 to study the physiological effect of the growth retardant paclobutrazol (PBZ) and its impact on yield of peanut plants. PBZ was applied as foliar spray at 50, 100, 150, 200 and 250 ppm in comparison with water spray as single foliar spray at 15 DAE, 25 DAE and as a double spray at 15 and 25 days after emergence (DAE). The results established that application of PBZ reduced plant height of peanut plants compared to control. The shortest peanut plants at different growth stages were produced due to application of PBZ at 200 ppm, however comparable with 150 ppm. Among different times of application, mean plant height was significantly reduced due to PBZ application at 15 DAE applications. Higher pod yield was recorded with 200 ppm, which was comparable with 150 ppm and significantly superior over control, 100 and 50 ppm. The increase in pod yield due to application of PBZ at 150-200 ppm either as single spray at 20 DAE or double spray at 20 & 30 DAE was due to a significant increase in number of pods. The reduced plant height, shorter inter-nodal length and increased thickness of young plant stem as well as the accelerated root formation are the significant advantages of the PBZ application, contributed to increase the flower to peg and peg to pod ratio thereby increase in the peanut pod yield. Foliar spray of PBZ increased the photosynthetic activity and water balance of peanut.

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Inheritance of SPAD chlorophyll meter reading and specific leaf area in two crosses of groundnut (*Arachis hypogaea* L.)

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Groundnut (*Arachis hypogaea* L.) is an important oilseed crop grown under wide range of environments in all over the world. Frequent occurrence of drought is the major limiting factor for adversely affecting the productivity of groundnut and hence genotypes with high water use efficiency (WUE) need to be developed. SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA) can be used as surrogate

traits for selecting for high WUE. The objective of this investigation was to elucidate the types of gene action governing the inheritance of SCMR and SLA in two crosses, NRCG 12568 × NRCG 12326 (cross I) and GKVK 4 × NRCG 12473 (cross II) by generation mean analysis. Five generations of each cross (P_1, P_2, F_1, F_2 and F_3) were evaluated for SCMR and SLA at 60 days after sowing (DAS). For SCMR, significant dominant effect and the duplicate dominance × dominance epistasis (*l*) were observed for the cross I and in the cross II, additive effect and additive × additive epistasis (*i*) were more predominant than dominance effects and duplicate dominance × dominance epistasis (*l*). For SLA, significant additive × additive epistasis (*i*) was registered for cross I and, for cross II significant additive effect and additive × additive epistasis (*i*) and dominance × dominance (*l*) epistasis were significant. Epistatic gene interactions would be more likely to interact with environmental effects than main effects *viz.*, additive and dominance. Predominance of dominant effect and duplicate dominant × dominant epistasis (*l*) for SCMR and SLA at 60 DAS suggested effective selection could be practiced in later generations.

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Utilization of wild *Arachis* species for peanut improvement

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Peanut (*Arachis hypogaea* L.), a tertaploid (AABB) with chromosome number 2*n*=4*x*=40, has a narrow genetic base, probably because of the bottlenecks associated with its origin. The genus *Arachis* contains 80 wild *Arachis* species and cultivated peanut is classified into nine sections. These wild species have enhanced levels of resistance/tolerance to multiple stresses, and provide important sources of genetic diversity to enhance the levels of resistance to key stresses and to broaden the genetic base of cultivated peanut. Peanut belongs to section *Arachis*, which also contains its tetraploid progenitor *A. monticola* Krapov. & Rigoni, and 29 wild diploid species that are cross compatible with peanut. However, utilization of these species for peanut improvement requires use of ploidy manipulation, bridge crosses, and ovule/embryo culture. ICRISAT genebank conserves 478 accessions of 48 wild *Arachis* species belonging to eight sections from six countries. For efficient utilization of diploid wild species, several amphiploids and autotetraloids have been synthesized at ICRISAT, Patancheru, India by using various A- and B- genome species to create new genetic variability for use in peanut improvement. These synthetics are being utilized in crossing programs with cultigens to develop pre-breeding populations/introgressions line (ILs) for utilization in crop improvement. Preliminary evaluation of two such populations have revealed sufficient genetic variability for resistance to diseases such as LLS, rust, and aflatoxin contamination and for morpho-agronomic traits. Development of such populations would provide sufficient variability to the breeders for use in peanut improvement programs to develop new cultivars with a broad genetic base.

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DArT/DArTseq based genetic mapping for identification of genomic regions controlling oil content in peanut

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Peanuts are primarily crushed for oil in countries such as China and India, and hence, development of new cultivars with high oil content is one of the major breeding priorities in these countries. In contrast, the peanuts in the United States are primarily used as edible products and lowering the oil content is

one of the main breeding objectives. In either case, the linked markers to the oil content are highly essential to deploy them in genomics-assisted breeding for developing cultivars with desirable oil content. In this context, one F_2 population (ICGV 07368 × ICGV 06420) was developed. Genotyping data have been generated for 53 simple sequence repeat (SSR) and 941 DArT/DArTseq polymorphic markers. Based on these marker data, a genetic map with 795 marker loci on 20 linkage groups covering a total map distance of 3,730 cM was constructed. The number of mapped loci ranged from 18-80 loci while length of the linkage groups ranged from 64-340 cM. This genetic map achieved a map density of 4.69 cM/loci. In parallel, the population has been phenotyped for oil content. Based on genotyping and phenotyping data, quantitative trait locus (QTL) analysis is in progress. The identified linked markers, after QTL analysis and marker validation, may be deployed in genomics-assisted breeding for developing superior peanut cultivars with desired level of oil content.

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Association between root and physiological traits in response to post flowering drought stress in groundnut (*Arachis hypogaea* L.)

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Root characters have been well established as drought tolerance traits in peanut. To investigate the relationship between root traits and physiological traits under post-flowering drought stress (PFD), raised bed experiments were conducted during summer seasons of 2013-14 and 2014-15 at the UAS, Dharwad. A factorial design considering the 299 RILS + two parents+ eight checks as Factor A, while Factor B consisted of non-stress (Irrigated) and stress conditions (45 days of PFD). Data were recorded for root traits such as root length, root to shoot ratio, number of root nodules and root dry weight after the imposition of stress. Data for physiological traits such as relative water content (RWC) and specific leaf area (SLA) were recorded at three consecutive time intervals (RWC I, II, III and SLA I, II, III respectively within period of stress imposition) while SPAD chlorophyll meter reading (SCMR) at 60 DAS. Large and consistent variation for both root and physiological traits existed among the RILs across years. Among the root traits, root length, root to shoot ratio and number of root nodules were positively interrelated in both the conditions across the years. Among the physiological traits RWC, SLA and SPAD have not shown significant correlation in the non-stress condition. During the stress condition RWC III was positively correlated with SCMR and negatively with SLA II and III in both years. Root length and root to shoot ratio were positively correlated with RWC III and SCMR, while negatively correlated with the SLA III. Among all the traits high RWC III, low SLA III, high SCMR, Increased root length and increased root to shoot ratio have contributed for high Drought Tolerance Index (DTI) and for maintenance pod yield under stress conditions.

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Candidate SNP markers for high oleate content in peanut

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As compared with its normal oleic counterpart, high oleic peanut has a longer shelf life and considered healthier for human and more suitable for biodiesel production. Hence, high oleic acid has become a major breeding objective of peanut. The role of *FAD2A* and *FAD2B* genes in conditioning oleate content in peanut has been well documented, however, there are some reports indicating the possibility that

other genetic factors also may be involved. A Super-BSA strategy was therefore utilized to identify candidate SNP markers for high oleate content in the cross Huayu 31× FB4. Made up of 42 F_2 individuals each, high and low oleate bulks had 74.328%-81.442% (average: 77.02%) and 20.641%-39.496% (average 35.22%) oleate, respectively. Totally 53,144 SLAF tags were obtained after sequencing the bulks and the parents. The overall average depth was 195×. Only 2,987 (5.62%) SLAF tags were polymorphic. 385 markers with clear parental origins were chosen for further association analysis using the SNP-index algorithm. 0.5302 was selected as the threshold value for Delta (SNP-index), as over 95% of the markers analyzed were found to have a lower value. Twenty SNPs with a Delta (SNP-index) value higher than 0.5302 were therefore identified as candidate markers for high oleate content in peanut. Hopefully, the A and B genome sequence information will be some help in elucidating the relationship between the SNPs and *FAD2A/FAD2B*, testing the candidate SNP markers in larger populations and isolating of the gene(s) of interest.

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Identifying SSR markers linked to TSWV resistance in peanut cultivar, Florida-EP^{TM}'113'

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Spotted wilt caused by tomato spotted wilt virus (TSWV) is one of the major diseases affecting peanut (*Arachis hypogaea* L.) production in the southeastern USA. Occurrence, severity and symptoms of spotted wilt disease are highly variable from season to season making it difficult to efficiently evaluate breeding populations for resistance. Molecular markers could overcome this problem and allow selection of resistant lines regardless of seasonal conditions. The objective of this study is to identify molecular markers linked to TSWV resistance in peanut cultivar, Florida-EP^{TM}'113' through genetic mapping. A total of 163 F_2 progeny derived from a cross between Florida-EP^{TM}'113', a TSWV resistant cultivar and Georgia Valencia, a susceptible cultivar. The $F_{2:3}$, $F_{2:4,}$ and $F_{2:5}$ populations were phenotyped by visual rating and/or immunostrip test. The Immunostrip results confirmed that symptomatic plants were infected by TSWV and many asymptomatic plants exhibited a positive immunostrip reaction, indicating that immunostrip test is a more sensitive method for TSWV phenotyping since asymptomatic, but infected plants can be identified. A total of 60 SSR markers flanking a known QTL for TSWV resistance were screened and 12 polymorphic markers were then used to genotype the whole F_2 population. The results showed that seven markers at linkage group A01 were linked with TSWV resistant QTL region, which was the same know QTL region identified previously. Fine mapping will be conducted by using F_6 population to identify markers closely linked to spotted wilt resistance.

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An international initiative to conduct comprehensive genome-wide association studies (GWAS) for an array of agronomic traits in peanut

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Advances in peanut genomics and natural variation present in germplasm collections offer opportunities for identification of superior alleles and trait-linked markers for rapid crop improvement. Although a few informative markers have been identified for some traits using bi-parental mapping population, genome wide scanning of germplasm collections can provide more reliable trait-linked genomic regions at the highest genetic resolution. Therefore, an international initiative has been started to undertake a comprehensive genome-wide association studies (GWAS) in three different germplasm sets i.e. the reference set (300 genotypes) consisting of global mini core collection (184 accessions) of ICRISAT, the U.S. mini-core collection (112 accessions) and the Chinese mini-core collection (298 accessions). The phenotypic data have been collected for 22 to 50 traits including resistance to diseases, aflatoxin contamination, tolerance to drought and salinity, and several agronomic (yield, 100-seed weight) and nutritional quality traits in several seasons at multiple locations. This initiative plans to compile and curate available phenotypic data, generate high-density genotyping data based on sequencing, conduct comprehensive statistical analysis, estimate diversity features and undertake GWAS for traits of importance to breeders using all three germplasm sets. In summary, this initiative is expected to provide: (i) sequence-based markers and haplotypes associated with traits of agronomic importance, (ii) accessions with superior alleles for their use as donors in breeding programs, and (iii) breeder-friendly databases for phenotyping and genotyping data. Additionally, the collaborative spirit of peanut research community will add momentum to peanut improvement.

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In Vitro evaluation of biocontrol agents against stem rot (*Sclerotium rolfsii*) and dry root rot (*Rhizoctonia bataticola*) of peanut

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Peanut is an important oil seed crop grown in India. Its cultivation in A.P is in an area of 11.26 lakh ha with a production of 8.99 lakh tonnes and productivity of 800 kg/ha. Stem rot (*Sclerotium rolfsii*) and dry root rot (*Rhizoctonia bataticola*) are destructive diseases of peanut causing severe production loss. Fungicidal control is uneconomical due to their soil borne nature. Biological control is only economical. Potentiality of nine native isolates of *Trichoderma* and *Pseudomonas* were evaluated by dual culture technique. Mycelial disc of five mm diameter of seven days old cultures of *Trichoderma* isolates and pathogens were placed on the opposite of the Petri plates at equal distance having PDA media. *Pseudomonas* isolates were streaked in square shape at the center of the plate 24 h before inoculation of pathogen. Plates were incubated at 27^{o}C. Hexaconazole 5% EC @ 2 ml/L was used as chemical check. All treatments were randomized thrice in completely randomized design. Percent inhibition of *R. bataticola* and *S. rolfsii* were ranged from 72.1 to 100.0 % and from 65.9 to 100 % respectively among all treatments. Significant inhibition of *R. bataticola* and *S. rolfsii* were achieved by *Pseudomonas fluorescence* PF 1 and it was at par with chemical check hexaconazole. Next best inhibition was recorded by *Trichoderma harzianum than T. viride* and *T. asperellum.* These two native bio control isolates were proved to be the best alternative to uneconomical chemical control.

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Construction of genetic linkage map and QTL analysis for yield and WUE related traits based on SSR markers for cultivated groundnut (*Arachis hypogaea* L.)

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Cultivated groundnut is an important oilseed crop, being grown more than 100 countries and it is ranking among the top five oilseed crops in the world. Although thousands of SSR markers have been developed for groundnut, very few genetic linkage maps based on cultivated × cultivated type have been published to date. The objective of this study was to construct a genetic linkage map and QTL analysis for yield and WUE related traits of cultivated groundnut using SSR markers. Based on $\Delta^{13}C$ value, SLA and SCMR, contrasting genotypes *viz.*, GKVK 4 (Low $\Delta^{13}C$) and NRCG 12473 (High $\Delta^{13}C$) were selected and 422 SSR primers were used to screen the polymorphism level between the parents. The F_2 mapping population was developed by crossing GKVK 4 × NRCG 12473. Among 422 SSR primers, 194 primers showed polymorphism between the parents, in that 13 primers could not differentiate one of the parental type and its heterozygote. Thirty five primers did not amplify for either one or both parents and 52 primers were not amplified in both parents. For construction of genetic linkage map and QTL analysis for yield and WUE related traits, currently genotyping work is under progress by using F_2 mapping population and in near future the same mapping population will be advanced to F_8 generation and additional primers will be added to the linkage map / QTL map.

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DArT/DArTseq based genetic mapping for identification of genomic regions for different fatty acids which control oil quality in peanut

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Peanut oil, a mild tasting vegetable oil is often used in Chinese, South Asian and Southeast Asian cuisine, both for general cooking, and in the case of roasted oil, for added flavor. Its major component fatty acids are oleic acid (46.8% as olein), linoleic acid (33.4% as linolein), palmitic acid (10.0% as palmitin) and others. With a perspective of health advantage for human consumption and longer shelf life for the peanut trade, it is desirable to develop peanut cultivars with the right combination of different fatty acids. In this context, one F_2 population derived from ICGV 06420 × SunOleic 95-R cross has been developed. This population has been genotyped using Diversity Arrays Technology (DArT) and DArTseq marker genotyping platform. Marker genotyping data have been generated for 1,725 (655 DArT and 1,070 DArTseq) loci of which 1,386 marker loci could be mapped on 20 linkage groups spanning a total map distance of 1,388.50 cM with an average of 1.0 cM/loci map density. The number of mapped loci ranged from 13 (Ah16) to 281 (Ah01). Length of the linkage groups varied from 25.59 cM (Ah18) to 171.42 cM (Ah02) while density among linkage groups ranged from 0.38 (Ah01) to 3.17 (Ah14) cM/loci. In parallel, fatty acids profiling is being undertaken on the population. Quantitative trait locus (QTL) analysis by using above mentioned genotyping and phenotyping data is expected to identify QTLs for different fatty acids. Major QTLs, if any, for the fatty acids profiled, after validation on different germplasm sets may be used as candidate QTLs for molecular breeding for developing peanut cultivars with varying combinations of different fatty acids.

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Proteome analysis of peanut gynophores and early swelling pods

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Cultivated peanut is one of the most important oil crops in the world, which flowering and forming peg (gynophore) in aerial, after gynophore penetrating into soil, the pod developed underground in darkness. Thus far, the molecular mechanism of geocarpy of peanut is poorly understood. To gain insight into the mechanism of this biological process, the comprehensive protein profiles of the aerial grown gynophores, subterranean un-swelling gynophores and gynophores with early swelling pod were analyzed by combining 1 DE with nanoLC-MS/MS approaches. A total of 2766, 2518 and 2280 proteins were identified at each stage, respectively. Among the identified proteins, 69 were involved in gravity response, light and mechanical stimulus, hormone synthesis and transport, calcium transport and signaling. Integrated analysis of proteome and transcriptome data led to the identification of 91 genes which were specifically or abundantly expressed in aerial gynophores at both mRNA and protein levels. Thirty-five specific or high abundant proteins were identified in subterranean un-swelling gynophores. We obtained 26 specific or high abundant proteins in early swelling pods. These results provided a global view at the protein level for understanding the underlying mechanism of peanut pod development.

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Development of SNP markers associated with resistance to Northern Root-knot nematode disease in cultivated peanut

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Northern root-knot nematode disease (Northern RKN) is one of serious soil-borne diseases affecting peanut production in China. One of pathways controlling Northern RKN disease is to develop and grow resistant varieties. There is a significant need to identify DNA markers linked to resistance to this disease for MAS breeding. A specific locus amplified fragment sequencing (SLAF-seq) technology and the bulk segregant analysis (BSA) approach were used to analyze the two population parents and resistant/susceptible DNA pools. The resistant DNA of the 30 most-resistant individuals from the F_2 population was combined to form a 'resistant DNA pool' and the same way for a 'susceptible DNA pool'. In total, 62,188 SLAF tags were obtained and 2,486 SNPs were polymorphic between the two parents. Fourteen SNPs were found to associate with disease resistance. Confirmation of tightly linked SNP markers is in progress. The results will be very useful for developing resistant cultivar through MAS.

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Development of late leaf spot and rust tolerant genotypes from TMV 2 and JL 24 by marker assisted backcross breeding in groundnut

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Foliar diseases like late leaf spot (LLS) and rust cause severe loss in the quantity and quality of the yield in groundnut. Development of foliar disease resistant genotypes, especially from the varieties that are already under commercial cultivation, but are susceptible to LLS and rust, is a promising approach in resistance breeding. The QTL and markers identified to be linked to LLS and rust resistance would hasten the selection scheme in the breeding program. TMV 2 and JL 24 released during 1940 and 1978, respectively for cultivation are still popular, except for their disease susceptibility. They were crossed to LLS and rust resistant genotypes like GPBD 4 (a released variety), ICGV 86699 (interspecific derivative), ICGV 99005 (interspecific derivative) and a second cycle derivative involving synthetic tetraploids. The F_1s were selected based on the allele type at LLS and rust resistance-linked markers. Three cycles of backcrossing was attempted, and a few homozygous plants were identified from the BC_3F_2 from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005. Selected BC_3F_3 families were highly resistant to LLS and rust, and they carried resistant allele at linked markers like IPAHM103 and GM2301. These lines were *on par* with the recurrent parent (JL 24) for test weight, SMK and yield. The background genome recovery in a selected family (JG_BC_3F_3_18) of JL24 × GPBD 4 was up to 86.6% when checked with 30 polymorphic transposable element (TE) based markers. Currently, BC_3F_4 lines are being evaluated in larger plots for productivity and disease resistance.

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The developmental oil mobilization of the peanut seed transcriptome

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The peanut seed transcriptome was analyzed using Illumina sequencing techniques. We produced 53,924,092 clean reads from four libraries, which corresponded to 4.85 Gb total nucleotides. These reads were assembled into 59,236 unique sequences. Differential mRNA processing events were detected for most of the peanut genes and found that 15.8% and 18.0% of the genes were differentially expressed between high-oil peanuts and low-oil peanuts at 30 DAF and 50 DAF, respectively. Over 1,500 unigenes involved in lipid metabolism were identified, classified and found to participate in FA synthesis and TAG assembly. There were seven possible metabolic pathways involved in the mobilization of oil during seed development. This dataset provides the most comprehensive sequence resource available for the peanut plant and will serve as the foundation for a system biology approach to the understanding of the oil mobilization in oil crops.

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Molecular characterization of five lines and three commercial varieties of peanut (*Arachis hypogaea* L.)

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Although the Mexican government does not require molecular characterization of candidate varieties for registration, the International Union for Plant Registration (IUPR) demands plant information using molecular markers. Therefore, in this research, five experimental lines and three commercial varieties of peanut (*Arachis hypogaea* L.) were characterized in order to corroborate genetic differences among materials. Sixteen ISSR (Inter Simple Sequence Repeats) were used. Among them ISSR2 produced 17 bands, with 25% of amplification through tested materials. According to Dice similarity coefficient (DS), 06-06Ch and 18-06CH were the most similar populations (DS = 0.91), while NC-17UACH and 4-06CH were the most distinct materials (DS = 0.79). Factorial analysis using Principal Coordinates (PCA) allowed grouping the genotypes into four groups: CECH and 4-06CH in group I; 18-06CH, 06-06CH, and NC-17AUCH made up group II; "Criollo rastrero de Cuauchi" formed group III; while "Rio Balsas" and "Matón Criollo de Cuauchi," formed group IV. Obtained results corroborated that new peanut lines are genetically distinct of commercial varieties.

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Screening for early maturing germplasm and attributing characteristics to identify associated SNPs in cultivated peanut

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Peanuts are grown under multi-cropping system in the vast peanut-producing regions of China. Early maturing peanut varieties are necessities due to limitation imposed by frost-free period, especially for the summer sowing directly after harvesting of winter crops in Northern China. In present study, 320 peanut cultivars were selected from landraces, breeding parents, released varieties of China and core accessions from different countries and used for early maturing germplasm screening, and further, for associated SNPs identifying by tGBS (tunable genotyping by sequencing). Five characteristics including days between sowing and flower initiation, days between flower initiation and 50% flowering, days between 50% flowering and 80% flowering, days between 50% flowering and 25 flowers, days between 50% flowering and 70% mature pods, and average daily flowering numbers in the first 10 days of flowering were investigated and their correlations with pod maturity were analyzed in 5 different harvesting stages. Dynamic accumulations of pod mass per plant were also estimated on the bases of harvestings in every 5 days during the period of 85 days to 130 days after sowing. Eleven early maturing germplasm lines were screened out and the characteristics attributed to early maturity were selected by correlation analysis. Associated SNPs to the parameters were identified by tGBS.

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Cloning, expression and evolutionary analysis of peanut *HIR* gene

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Hypersensitive-induced reaction (*HIR*) gene belonging to PID family plays important roles in plant hypersensitive response (HR) and results in localized cell death and formation of necrotic lesions. In this study, partial sequence of peanut *HIR* gene was obtained from transcriptome sequences, and the full length cDNA and genomic sequence of peanut *HIR* gene was acquired by RACE amplification. Bioinformatics analysis showed that *AhHIR* is conserved in different plant species. PLAM software analysis demonstrated that *HIR* gene may be strongly selected by purify selection during evolution. The quantitative RT-PCR results showed that the expression of *AhHIR* was significant reduced in leaves upon cold stress for four hours and the expression level was gradually increased after longer treatment. The pathogenic bacteria infection also caused a decrease in expression level of this gene. These results provided useful information for further functional study of *AhHIR* in both biotic and abiotic stress tolerance.

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Molecular cloning, expression and evolution analysis of type II *CHI* gene from peanut (*Arachis hypogaea* L.)

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Chalcone isomerase (CHI) plays critical roles in plant secondary metabolism, which is important for interactions between plants and their environment. CHI genes are widely studied in various higher plants. However, little information about CHI genes is available in peanuts. In the previous study we cloned and analyzed peanut type I CHI gene. In this study, based on conservation of CHI gene family, we cloned peanut type II CHI gene (*CHI II*) cDNA and genome sequence. The amino acid sequence of peanut CHI II was highly homologous to type II CHI from other species. qRT-PCR results showed that peanut *CHI II* mainly expressed in roots; however, peanut *CHI I* is mainly expressed in tissues with high content of anthocyanin. Gene duplication and gene cluster analysis indicated that *CHI II* gene was derived from *CHI I* gene approximately 65 million years ago. Gene structure analysis results supported the hypothesis that *CHI II* gene was derived from *CHI I* by the insertion of an intron into the first exon. Moreover, no positive selection pressure was found in *CHI* genes, but 32.1% of sites were under neutral selection, which may lead to mutation accumulation and fixation during great changes in environment.

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Development of introgression lines and advanced backcross QTL analysis for disease resistance, oil quality, and yield component traits in peanut

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Ploidy difference between wild *Arachis* species and cultivated genotypes hinder transfer of useful alleles for agronomically important traits. To overcome this genetic barrier, several synthetics have been developed at ICRISAT. Furthermore, two synthetic amphidiploids viz., ISATGR 1212 (*A. duranensis* ICG 8123 × *A. ipaensis* ICG 8206) and ISATGR 265-5A (*A. kempff-mercadoi* ICG 8164 × *A. hoehnei* ICG 8190) have been used to generate two advanced backcross (AB) populations. The AB populations namely AB1 (ICGV 91114 × ISATGR 1212) and AB2 (ICGV 87846 × ISATGR 265-5A) have been genotyped with DArT and SSR markers. Based on these marker genotyping data, 258 (253 DArT and 5 SSR) and 1043 (1034 DArT and 9 SSR) loci have been mapped on 20 and 19 linkage groups covering a total map length of 1415.7 cM and 1500.8 cM with map density of 5.49 and 1.44 cM for AB1 and AB2 populations, respectively. These populations have also been phenotyped for disease resistance (late leaf spot, rust and peanut bud necrosis), oil quality (oleic and linoleic acid ratio, saturated and unsaturated fatty acids), and yield components (pod yield, 100 seed weight, sound mature kernels and shelling percentage). Quantitative trait locus (QTL) analysis using above mentioned genotyping and phenotyping identified 15 QTLs (PV 6.68-14.84%) in AB1 population while 34 QTLs (PV 6.9-67.8%) in AB2 population for disease resistance, oil quality and yield component traits. The AB-QTL approach facilitated simultaneous identification of QTLs and introgression of wild genomic regions associated with traits of interest into the cultivated gene pool of peanut.

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Harnessing natural genetic variation for trait mapping in peanut

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Peanut research community has witnessed the speedy development of genomic resources including large number of molecular markers (SSRs, SNPs and DArTs) and recent availability of the genome sequences of two diploid progenitors. With an objective to harness natural variation present in germplasm (cultivated, landraces and wild species/synthetics), a range of genetic resources such as bi-parental, nested-association mapping (NAM) and advanced backcross (AB) populations, multi-parent advanced generation intercross (MAGIC) lines, the 'reference set', the 'minicore collection' and a training population have been developed. Use of the above genetic and genomic resources together with phenotyping data for a range of traits resulted in development of several genetic and consensus genetic maps, and identification of linked markers through linkage (including AB-QTL) and association mapping approaches for foliar disease resistance, drought tolerance related traits, oil and nutritional quality traits and several other important agronomic traits including pod yield. Functional genomics and sequence-based trait mapping approaches have also been initiated for gene discovery and breeder-friendly marker development. Molecular markers linked to rust, late leaf spot, and high oleate traits are being employed in breeding program using marker-assisted backcrossing and gene pyramiding approaches for increasing precision and efficiency in selection and shorten the varietal developmental duration. Besides, to improve complex and agronomically important traits, another modern breeding approach namely genomic selection has also been initiated. Availability of genome sequence is expected to accelerate trait mapping and eventually crop improvement.

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Genetic mapping and QTL analysis of agronomic traits in cultivated peanut (*Arachis hypogaea* L.)

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Peanut (*Arachis hypogea* L.) is one of the most important economic crops. Improved agronomic trait is a driving force for peanut genetic studies and breeding. In the present study, a recombinant inbred lines (RILs) population containing 251 lines from a cross between Silihong (var. *fastigiata*) and Jinonghei 3 (var. *hypogaea*) was established, and 192 SSR primer pairs were used to construct a genetic linkage map. The map included 117 markers distributed on 22 linkage groups and covered 1031.6 cM with an average distance of 8.8 cM between adjacent markers. Of these 22 linkage groups, 19 were anchored on 15 chromosomes. Based on the newly constructed map of tetraploid peanut, we performed QTL mapping of 18 agronomic traits from the RIL_8 and RIL_9, grown in Baoding and Handan in 2012 and 2013, respectively. A total of 57 QTL related to 16 agronomic traits were detected on 11 chromosomes and 2 unknown groups, explaining 3.81% to 53.37% of the phenotypic variation. Eight QTL of these agronomic traits were identified under two ecosystems. Two QTL for height of main stem and seed thickness were detected in Baoding and Handan in 2012 and explained 6.36% and 10.12% of the phenotypic variance. Six QTL were identified for seed length, seed width, seed thickness, seed weight per plant, shelling percentage per plant and total branching number in Baoding and Handan in 2013 and explained 5.7% to 53.37% of the phenotypic variance. These stable QTL for agronomic traits could be used for marker assisted selection.

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Identification of quantitative trait loci for important agronomic traits in cultivated peanut (*Arachis hypogaea* L.)

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Many important agronomic traits of cultivated peanut are quantitative traits that are controlled by multiple genes. Identifying quantitative trait loci (QTL) for these traits is an important work in peanut quantitative genetics research. A mapping population consisting of 142 $F_{6\text{-}7}$ individuals derived from the cross between 04D893 and 79266 was used to establish a genetic linkage map of cultivated peanut; 92 marker loci were mapped into 18 linkage groups covering a total distance of 516.7cM. Fourteen agronomic traits associated with leaf, plant type, pod and kernel in cultivated peanut were identified by the method of Inclusive Composite Interval Mapping. Twenty-eight QTLs and 22 QTLs for these traits were identified in 2011 and 2012, respectively. Seven QTLs associated with 5 traits were identified stably in two years as following:

(1) Qll-14-1 was for leaf length, with 13.82% phenotypic variance explained (PVE), the mean genetic distance (MGD) between the F92 marker and the QTL is 2.5cM.
(2) Qsla-11-1 was for single leaf area with 13.98% PVE, the MGD between the ARS376 marker and the QTL is 0.7cM.
(3) Qmsh-11-1 and Qmsh-11-2 were for main stem height, with 13.83% and 63.10% PVE, the MGD between the ARS205 marker and the former is 0.9cM, the MGD between the ARS376 marker and the latter is 1.2cM.
(4) Qtbn-4-1 and Qtbn-6-1 were for total number of branch, with 8.31% and 10.48% PVE, the MGD between the marker F60 and the former is 0cM, the MGD between the ARS318 marker and the latter is 0.5cM.
(5) Qtps-14-1 was for thickness of pod shell, with 9.27% PVE, the MGD between the F167 marker and the QTL is 2.0cM.

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Selection of interspecific lines at the first backcross generation for the runner market in Brazil

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The development of leaf spots resistant, high oleic runner cultivars is one of the main demands from the peanut growers in Brazil. Since 2011, 12 plants of the BC_1F_3 population [*Arachis hypogaea* cv. Runner IAC 886 x (*A. ipaënsis* x *A. duranensis*)^{4x}] and lines derived from them are under evaluation for leaf spot resistance, production and oil quality in the peanut breeding program at EMBRAPA. Two partial resistant lines and 42 runner lines were selected among these interspecific progenies. The partial resistant lines were used for the generation of new breeding populations; four high oleic acid content lines in F_4 were selected. Evaluation of the 42 runner lines detected significant difference in production (P=0.03, CVg/CVe = 2.04) compared to the recurrent parent (4253.6 kg.ha^{-1}). The lines with THE highest production were derived from progenies LPM 12, LPM 20 (line 2012-33, 7759.9 kg.ha $^{-1}$) and LPM 22. The potential for the use of wild *Arachis* species in breeding has been noticed since the 1970s, and recent reports detected the contribution of wild species for several agronomic traits. However, an intensive backcrossing program is the most common approach to recover production and market acceptable traits. The variability observed for agronomic traits in the progenies and lines derived from these BC1 plants allowed us to select lines with high production and potential for cultivar release. The generation of breeding populations using these selected genotypes is an alternative approach to the traditional backcrossing programs to allow section of transgressive resistant and highly productive genotypes.

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