

Abstract Book



AAGB-2009

*Fourth International Conference of the
Peanut Research Community*

on

**Advances in *Arachis* through Genomics
and Biotechnology (AAGB-2009)**

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West and Central Africa Regional Peanut Workshop

ICRISAT-Bamako, Mali, Africa

19-22 October 2009

Organized by



**International Crops Research Institute
for the Semi-Arid Tropics**

in collaboration with



Peanut Science Council, USA



Institut d'Economie Rurale du Mali (IER)



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***Theme 1:
Genetics, Allelic Diversity and
Germplasm Enhancement***

Use of marker-assisted selection to develop disease resistant cultivars with high O/L ratio

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Close cooperation between conventional plant breeders and molecular geneticists will be needed to efficiently and effectively utilize modern genetic tools in the development of peanut cultivars. We have used this approach to develop molecular markers for resistance to the peanut root-knot nematode and molecular markers for both alleles responsible for high oleic fatty acid content. We are currently utilizing these markers in an accelerated back cross breeding program to develop a high oleic Tifguard. Tifguard, a peanut cultivar released in 2007, has near immunity to root-knot nematode, high resistance to tomato spotted wilt virus (TSWV), and moderate resistance to late leaf spot. However, its oil composition is within the normal O/L range. We hybridized Tifguard with two high oleic cultivars, Georgia 02C and Florida 07. We then used molecular markers to test and select the appropriate F₁ plants and used these as parents to develop the first and second backcross generations. This accelerated backcross breeding program with marker assisted selection should result in the development of high oleic Tifguard in 26 months.

Current efforts in conventional and molecular breeding of groundnut in Ghana

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Groundnut (*Arachis hypogaea* L.) is the most important grain legume in Ghana and is widely cultivated in all agro-ecological zones. Until recently, groundnut breeding has comprised solely of introductions of advanced breeding lines from ICRISAT. In 2005, four (4) late maturing improved varieties resistant to important diseases such as rosette and *Cercospora* leaf spot were released and recommended for planting in Ghana. A recent screening of local accessions in Ghana has resulted in identifying accessions possessing high oil and high nutritional quality, and a high oleic/ linoleic ratio which indicates potential for better storability. However, these local accessions have inherent low yields and are susceptible to diseases such as Rosette and *Cercospora* leaf spot. Current research is geared towards improving these local accessions through the introgression of rosette resistant genes into the local accessions using conventional and molecular breeding methods. Since molecular markers and genetic linkage maps are pre-requisites for molecular breeding in any crop species, as a first step, available microsatellite primers developed at ICRISAT and Tuskegee University are being screened to find SSR markers that show polymorphism between the susceptible and resistant parents. If sufficient polymorphic primers are found, they will be used to screen F2 population and genetic linkage map will be constructed. The present study also reports the diversity among the local accessions using microsatellite markers.

A Survey of SSR Polymorphism in 112 Indian Groundnut Cultivars

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Occurrence of limited DNA polymorphism in cultivated groundnut (*A. hypogaea* L.) has so far been a major bottleneck in the using marker aided breeding techniques. Recently a good number of SSR markers have been developed and become accessible which has opened up new possibilities. In India about 180 varieties of groundnut have so far been released, which have been developed through breeding procedures ranging from mass selection to hybridization followed by different approaches of selection. Therefore, the extent of DNA polymorphism was surveyed in 112 groundnut varieties comprising four Valencia 27 semi-spreading, 24 spreading and 57 Spanish types by using 13 SSR makers reported to be polymorphic in groundnut by earlier workers. For 57 erect cultivars, the number of bands was in the range of 2 to 7. In the spreading and semi spreading types, the size of the amplicons was in the range of 60 to 323 bp. In all 50 bands were produced with an average frequency of 4 bands per primer. The SSR primer index (SPI) of each primer was calculated and some primers seemed to be highly informative. Clustering of the molecular data revealed two major groups for erect, and spreading and semi-spreading types. Altogether four clusters formed two major clusters each for erect, and spreading and semi-spreading types. The genetic distance between the varieties was short. It was thus concluded that despite having some degree of polymorphism, the genetic diversity in groundnut at molecular level was inadequate for association of markers with specific traits.

Genetic diversity of peanut genotypes in mini core collection using SRAP-RGA DNA markers

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Sequence-related amplified polymorphism (SRAP) DNA marker system is useful in targeting coding sequences in plant genome through amplification of open reading frames (ORFs). The majority of plant disease resistance gene proteins belong to nucleotide binding site-leucine rich repeat (NBS-LRR) class. Resistance gene homologs (RGHs) could be amplified using degenerate primers designed based on the conserved motifs within NBS domain. We have identified 589 unique RGH sequences, from which specific RGH primers were designed based on the divergent RGH sequences. In this study, combinations of SRAP and RGH primers were used to amplify NBS-based R genes in peanut. Seven hundred thirty two SRAP-RGH primer pairs were screened using four genotypes, and 40% of those primer combinations could detect polymorphism. Twenty-six primer pairs were used to amplify 24 accessions selected from the mini core collection and produced 293 polymorphic bands in total of 431 bands. Cluster analysis showed that wild species could be distinguished from those cultivated genotypes

Genetic diversity in Global Valencia peanuts (*Arachis hypogaea* L. ssp. *fastigiata* var. *fastigiata*) using SSR markers

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Cultivated peanut, grown worldwide is a major oil producing legume crop. Valencia peanuts (*Arachis hypogaea* L. ssp. *fastigiata*) are grown in eastern New Mexico and west Texas for their in-shell market value. In the present study molecular characterization of 114 Valencia peanuts (78 accessions from US Valencia core and 35 accessions from global mini core and 1 control cultivars from ICRISAT) representing various geographical regions of the world was performed using microsatellite markers. Out of 100 primer pairs tested, 52 polymorphic primers amplified a total of 683 alleles. The number of alleles ranged from 2 to 28 per locus with a mean of 13.13 alleles per primer pair. Mean gene diversity and PIC value for all the alleles were 0.3496 and 0.2805, respectively. Based on Jaccard's coefficient of genetic similarity, accessions were clustered using unweighted pair group and the dendrogram was generated in the NTSYS-pc 2.20e. Two major clusters A and B sharing 30% similarity were obtained. Cluster A consisted of accessions predominantly from South American region. The cluster B consisted of an intermix of accessions from North America, South America, Africa, Asia and Caribbean regions. A single accession ICG6201 from the Caribbean region separated from rest of all the accessions and shared a similarity of only 10%. Principal component analysis (PCA) depicted two major clusters with few accessions scattered apart in minor groups. In our study 41.18% of variation was explained by the first three PCs. The genetic diversity observed in Valencia core and global mini core could be used in breeding Valencia cultivars.

Content of some nutrients in the core of the core of the U.S. peanut germplasm collection

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The usefulness of core collections of germplasm collections has been well established. The U.S. peanut germplasm collection was previously selectively reduced based on morphological characteristics to a mini core or “Core of the Core” collection composed of 112 of the 7432 accessions in the whole collection to make it more efficient for study. Of these samples, 108 were available from one growing location in the same year and were therefore exposed to one set of environmental conditions where in genetic variability could also be examined. These samples were analyzed for total and individual amino acid content, fatty acid content, tocopherols and folic acid content. These data provide a starting point for establishing nutrient composition within these accessions and provide early indication of currently important characteristics in these lines which might be suited for use in random breeding initiatives.

Genetics of traits related to Water Use Efficiency and Transpiration rate in Groundnut (*Arachis hypogaea* L.)

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Development of drought tolerant genotypes is the need of the day. In this regard SPAD chlorophyll meter reading (SCMR), Specific leaf area (SLA), $\Delta^{13}\text{C}$ and $\delta^{18}\text{O}$ stable isotope discrimination were used as surrogates to identify genotypes for drought tolerance in F_2 generation of an elite cross NRCG-11915 x NRCG-12326 in groundnut. There was significant difference for all the traits. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) estimates were high for most of the traits including pod yield, kernel yield and SLA coupled with high broad sense heritability and genetic advance as per cent mean. This reflects the presence of considerable heritable variation and the influence of additive gene effects in the expression of these traits.

SCMR had inverse relationship with SLA and $\Delta^{13}\text{C}$. Pod yield exhibited highly significant positive association with kernel yield, shelling percentage and SCMR. Where as kernel yield also recorded highly significant positive association with SCMR and shelling percentage and significant negative association with SLA. Inter generation correlation studies for F_2 and F_3 generations showed highly significant positive correlation for SLA and kernel yield across the generations coupled with moderate narrow sense heritability indicating the possibility of simultaneous improvement of these traits by selection. So, phenotypic selection could be practiced at early generation to improve SLA and SCMR for increased yield.

The trait $\Delta^{13}\text{C}$ was normally distributed in F_2 generation indicating their polygenic nature of inheritance. Stable isotope $\delta^{18}\text{O}$ showed skewed distribution in F_2 towards low $\delta^{18}\text{O}$. It indicates their polygenic or multiallelic nature of inheritance. Relationship of SLA and SCMR with other traits and nature of inheritance of carbon and oxygen isotopes could be used to identify genotypes with high WUE and high transpiration rate in segregating generation.

***Theme 2:
Genome Resources and
Genome Analysis***

Reference genetic resources for peanut

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Financial support: Generation Challenge Program Tropical Legumes 1 and host institutions.

Cultivated peanut is an allotetraploid ($2n = 4 \times = 40$ chromosomes) with two genome types, A and B, which are found separately in the diploid wild species of the *Arachis* section. Cultivated peanut was originated via hybridization of two diploid wild species, probably *A. duranensis* (A-genome) and *A. ipaënsis* (B-genome), creating a hybrid that would initially have been sterile. Most probably only one, or a few, spontaneous chromosome duplication events restored fertility and gave rise to tetraploid cultivated peanut. This genetic bottle-neck, coupled with reproductive isolation from its wild diploid relatives has led to a limited genetic diversity, and has hampered the development of peanut genetic and genomic resources. We have worked towards the production of reference genetic maps for peanut research and breeding. With this aim we have created three recombinant inbred line populations each of about 100 lines: one diploid population created from the most probable A-genome donor of cultivated peanut (*A. duranensis*) crossed with a close relative, *A. stenosperma*; one diploid population created from the most probable B-genome donor to cultivated peanut (*A. ipaënsis*) crossed with a close relative, *A. magna*; and a tetraploid population derived from cultivated peanut crossed with a synthetic amphidiploid (*A. ipaënsis* x *A. duranensis*)^{4x}. Wild species were used in all of the crosses so as to create populations with high levels of polymorphism. This high polymorphism means a high percentage of candidate DNA markers are informative, thus facilitating the map's cross-referencing to other maps: genetic maps derived from purely cultivated crosses, genetic maps of other legumes, and a physical map of the A-genome of *A. duranensis*. At the moment the genetic maps based on the recombinant inbred lines are being constructed and the most informative lines being selected. DNA and seed from the lines will be available from the authors on request.

Development of genomic resources for cultivated groundnut (*Arachis hypogaea* L.)

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With an objective to develop large scale genomic resources in cultivated groundnut, efforts are being made at ICRISAT, in collaboration with its partners, to generate molecular markers like simple sequence repeats (SSRs) and Diversity Array Technology (DART). For instance, more than 250 novel SSR markers were developed either from genomic DNA libraries (Cuc et al. 2008, BMC Plant Biol 8:55; Gautami et al. submitted) or mining the gene sequences from aeschynomenoid/ dalbergoid and genistoid clades of the Leguminosae (Mace et al. 2007, Plant Science 174:51-60). Similarly a DART array comprising of 7600 features has been developed. These markers, in addition to those available in public domain and accessible through collaborators, are being/have been used to detect polymorphism between parental genotypes of four mapping populations namely TAG 24 × ICGV 86031, TAG 24 × GPBD 4, ICGS 44 × ICGS 76 and ICGS 76 × CSMG 84-1. In general, 6-10% polymorphism has been observed with the SSR markers tested in the above mentioned germplasm. Genotyping of polymorphic markers has facilitated development of genetic maps with moderate marker density (56- 165 per cross) for respective mapping populations (e.g. Varshney et al. 2009, Theor Appl Genet 118:729-739). Detailed QTL analysis for drought component traits as well as disease resistance, by using single marker analysis (SMA), composite interval mapping (CIM) and epistatic interaction analysis is underway. Nevertheless, one major QTL, contributing upto 54.4% phenotypic variation for rust resistance has been identified in TAG 24 × GPBD 4 population which has been validated also in different genetic backgrounds using diverse germplasm lines and mapping populations. In summary, such efforts on development of genomic resources such as SSRs, DARTs, genetic linkage maps and identification of QTLs will be of great help for facilitating molecular breeding and improve crop productivity of cultivated groundnut.

Development of microsatellite, single nucleotide polymorphism, and transposon markers for peanut breeding

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In order to construct a linkage map of peanut, we developed three kinds of markers, i.e., microsatellite, single nucleotide polymorphism (SNP), and transposon markers. Two F₂ mapping populations derived from crosses between “Satonoka” x “Kintoki” and “Nakateyutaka” x “Hachi-I-0311” were developed for the segregation analysis. “Satonoka”, “Nakateyutaka”, and “Hachi-I-0311” are valencia types, while “Kintoki” is a spanish type. Polymorphic analysis was performed with a total of 2771 microsatellite markers, including 1110 published markers and 1661 markers developed in our laboratory. Out of them, 321 and 144 showed polymorphism between “Satonoka” x “Kintoki” and “Nakateyutaka” x “Hachi-I-0311”, respectively. SNPs discovery has been performed by comparison of a large scale of expressed sequence tags obtained by high-throughput DNA sequencer, 454 FLX and Genome Analyzer. Large number of pseudo SNPs between orthologues inhibited discovering “real” SNPs between the mapping parents, and suggested necessary to develop a novel algorithms to mask those orthologus SNPs. Miniture-inverted transposable elements (MITEs), which are multi-copy nature in the peanut genome, could be used as DNA markers by designing primer pairs on the franking regions of the MITEs captured by a hybridization technique. Among 417 transposon markers, 110 and 59 were exhibited polymorphism between “Satonoka” x “Kintoki” and “Nakateyutaka” x “Hachi-I-0311”, respectively. With the polymorphic microsatellite, SNP, and transposon markers, we have constructed a tentative linkage map. The map will contribute peanut breeding through marker-assisted selection.

Development of two recombinant inbred lines populations for peanut genetic linkage map and QTL analysis

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Cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid species (AABB, $2n = 4x = 40$) that still lacks a “true” tetraploid genetic linkage map with all expected 20 linkage groups. Even though several maps have been developed, they were constructed using diploid or interspecific tetraploid populations. As a result, the application of marker-assisted selection to the improvement of the cultivated peanut has been hampered by lacking a road-map and by an inability to visualize genetic variation. In the southeastern U.S., tomato spotted wilt virus disease has become a major limiting factor for many peanut producers, while the control methods are limited. Both early (*Cercospora arachidicola*) and late (*Cercosporidium personatum*) leaf spot diseases are among the worst foliar diseases of cultivated peanut. Infection of peanut with *Aspergillus parasiticus* and consequent contamination with aflatoxin are a serious threat to human and animal safety. Our interest is to develop RIL (recombinant inbred line) mapping populations for genetic linkage map construction and QTL (quantitative trait loci) studies of interest traits. Two RIL populations have been developed from crosses of Tifrunner x GT-C20 and SunOleic 97R x NC94022. The populations were advanced to the F₄ by single seed descent. Individual plants were harvested and progeny rows were grown to produce the F_{4:7} RIL populations. The populations consisted of 248 individual lines for Tifrunner x GT-C20 and 354 individual lines for SunOleic 97R x NC94022. We have screened over 3,000 SSRs for polymorphisms among the parental lines and collected morphological traits and disease resistance for characterizing these two populations. These populations will be made available for the community and the collaborators for marker development and QTL studies.

A novel set of SSRs developed from BAC-end sequences

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Despite the availability of several thousand simple sequence repeat (SSR) primer pairs for cultivated peanut, exceedingly low rates of polymorphism constrain the number of useful markers. To address this deficiency we have mined DNA sequences from the ends of bacterial artificial chromosome (BAC) clones for additional novel SSRs. 4,448 BAC end sequences of *A. hypogaea* Tifrunner were obtained from 3784 BAC clones that were selected based on hybridization to peanut NBS-LRR disease resistance genes; these sequences yielded 142 new SSRs (RGH-SSRs) that met our criteria for SSR content and length. These same *A. hypogaea* BAC clones were fingerprinted to produce physical map contigs of regions of the peanut genome containing disease resistance gene homologs. In addition, we sequenced 25,000 randomly selected BAC clones of *A. duranensis*, resulting in 41,856 end sequences and 1392 SSRs that met criteria for length and content. A total of 1456 primer pairs were designed and tested for amplification on one tetraploid and one diploid accession. Approximately 80% of primer pairs yielded simple amplification products, allowing us to produce a subset of 1152 functional primer pairs that we are currently analyzing for polymorphism across a panel of cultivated *A. hypogaea* accessions.

Genetic mapping and synteny analysis allowed the identification of genome rearrangements in the allotetraploid *Arachis hypogaea*

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Cultivated peanut (*Arachis hypogaea* L.) is considered to be an allotetraploid AABB ($2n = 4x = 40$) originated from a recent and single hybridization event between two wild diploids. *A. duranensis* (AA) and *A. ipaensis* (BB) appeared to be the best candidates for the A and B genome donors, respectively. Recently, the development of synthetic amphidiploids using wild diploid species allowed overcoming the reproductive barrier between wild diploids and the cultivated species. The objectives of this study were to construct a wild x cultivated tetraploid genetic map using SSR markers and to analyse the synteny between the A and B genomes.

A synthetic amphidiploid, obtained from the cross between the most probable wild progenitors of the cultivated peanut (*A. duranensis*, *A. ipaensis*), was crossed to the Fleur11 variety. A population of 88 BC₁F₁ individuals was produced and genotyped with 277 polymorphic SSR markers. We mapped 298 loci in 21 linkage groups (LGs), spanning a total map distance of 1843.7 cM. We identified the homeologous LGs with 53 SSR markers that mapped on both the A and B genomes. We observed an overall good collinearity between each pair of homeologous LGs. However, three inversions of chromosome segment were pointed out between homeologous LGs a01/b01, a03/b03 and a09/b09, as well as a major translocation involving the LGs b07 and b08. The result of this study contributes to the comprehension of the structure of the A and B genomes and the broadening of the gene pool of the cultivated peanut.

Transcriptome analysis of wild *Arachis* under water stress

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Due to its high genetic diversity and adaptation to a range of environments, wild relatives of peanut (*Arachis hypogaea* L.) are an important source of genes for resistance to biotic and abiotic stresses that affect peanut and other crop species. For example, *A. magna* and *A. ipaënsis*, wild types BB diploid species, and *A. duranensis*, a wild type AA diploid species, show high adaptability to water stress. In the last few years, a cooperative initiative has generated tools for molecular breeding, and the incorporation of wild genes into cultivated peanut, including the development of functional genomics resources for wild *Arachis* species. In this context, water stress-responsive genes in the transcriptome of *A. magna* (accession KG30097) subjected to gradual water deficit were analyzed and 13 candidate genes were revealed. Those genes are up- or down-regulated exclusively in the stressed or control conditions and involved mostly abiotic stress. Some of these genes were further characterized through *in situ* hybridization and RT-qPCR in order to evaluate their expression profiles spatially and temporally and the potential role in the mechanisms of plant response. This is, to date, the first report on the analysis of wild *Arachis* transcriptome under abiotic stress. The information obtained in this study is a valuable resource for gene discovery, the characterization of new wild alleles, and for marker development.

The role of retrotransposons in evolution of the allotetraploid genome of cultivated peanut

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Retroelements are dynamic components of plant genomes and are important factors in the differentiation of and the sexual compatibilities between different species. We have focused one of our research lines on the isolation and characterization of retroelements in *Arachis* aiming to find out more about the genomic relationships between the most probable ancestors of cultivated tetraploid peanut. One of the major retroelements involved in the evolution of the different genomes seems to be a retrovirus-like Ty3-*gypsy* LTR retrotransposon, which we have named FIDEL. As revealed by fluorescent *in situ* hybridization (FISH), FIDEL is dispersedly distributed in the euchromatic regions of the chromosomes and preferably present in the A-genome. Copy number estimations showed about four times more copies of the element in the A- (~ 3000 copies) as compared to the B-genome (~ 800 copies), and phylogenetic studies indicated distinct evolution of FIDEL in the ancestor species of *Arachis hypogaea*. Databases of BAC-end sequences from BAC libraries of the A- and B-genome allowed us to identify conserved domains of other retroelements, which then have been cloned by PCR and used as probes in FISH experiments. By this we have detected CAIPORA, a middle-repetitive LTR-element, which is more prominent on B-genome chromosomes. In addition, in contrast to the elements with clearly distinct evolution in both genomes, the Ty1-*copia* element MATITA has a lower copy number and is characteristically located in the distal regions of chromosomes of the A- and B-genomes. Our results emphasize the role of retroelements in the evolution of the different peanut genomes and contribute to a better understanding of the divergence of the different species.

***Theme 3:
Abiotic/Biotic Stresses and Quality***

Phenotyping groundnut mini-core collection for resistance to *Aspergillus Flavus* and Aflatoxin contamination, Sadore, Niger

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Aflatoxin contamination of groundnut has gained global significance due to the deleterious effects on human and animal health. There is evidence that the level and duration of exposure to aflatoxin can result in acute and chronic symptoms of what? with severe consequences. Efforts have been made to identify and use genes that are conferring resistance to aflatoxin contamination in groundnut. Often these sources don't possess high levels of resistance and have been identified from a limited part of available global germplasm collection. The representative mini core collection (1% of entire collection) consisting of 184 accessions provided a gateway for accessing the global germplasm diversity.

A total of 180 mini-core accessions of groundnut and 4 controls 2 resistant varieties (55-437 and J11 and 2 susceptible varieties-Fleur11 and JL 24) were screened for resistance to infection by *A. flavus* and subsequent aflatoxin contamination at Sadore, Niger during the 2008 rainy season. Preliminary results showed a wide range of variations in infection by *A. flavus* (5.19 % (ICG-36) to 100 % (ICG-14482). Among the 180 mini core lines tested, 20 lines showed low levels of infection (< 10%). The mini core accessions also showed a large variation in Aflatoxin content (2.3 ppb for ICG-5195 to 4703.4 ppb for ICG-14482). Out of the 184 lines tested 13 showed less than 5 ppb aflatoxin content compared to the resistant checks (7.2 ppb for J11 and 11.1 ppb for 55-437).

Pods yield of the resistant accessions varied from 1.065 t/ha (ICG 11515) to 4.023 t/ha (ICG 12370). The haulm yields varied from 3.3 t/ha (ICG-10474) to 11.51 t/ha (ICG-12000)

In conclusion the mini-core showed a wide range of variation in both agronomic and aflatoxin contamination levels.

Disease resistance characterization in wild *Arachis*: microscopy, gene expression analyses and genetic mapping.

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Worldwide, diseases are important reducers of peanut (*Arachis hypogaea*) yield. Wild species harbor resistance against many pests and diseases, such as root-knot nematode and foliar diseases caused by fungi. In this work, an accession of *A. stenosperma* presented complete resistance to these diseases and nematode species. This same accession is the parent of an AA population to which an *Arachis* reference map was created. Late leaf spot (LLS), rust and nematode interaction in *A. stenosperma* were morphologically characterized by electron and light microscopy. It was found that the fungal diseases were suppressed at early stages of germination, and that nematode infection was also most prevented pre-penetration. The small number of nematodes that did penetrate were unable to set up feeding sites being surrounded by a hypersensitive reaction. In order to identify candidate genes related to response to nematodes, cDNA libraries were created and sequenced, generating eight candidate sequences, which have been validated and mapped. For *A. stenosperma* challenged with LLS, pyrosequencing is being used for identification of differently expressed genes. The reference genetic map was enriched with RGA-derived markers and markers derived from candidate genes. QTLs for components of resistance to LLS were identified. Phenotyping will take place with this population in order to identify QTLs for nematode resistance. Synteny with the genome of cultivated peanut will allow the more direct use of this information in breeding programs. Additionally, amphidiploids using *A. stenosperma* as a parent are being produced and their use will allow gene transfer to the cultivated species.

Comparative analyses of peanut expressed sequence tag libraries

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Large-scale single-pass sequencing of cDNAs from different plants has provided extensive reservoir for the cloning of genes, the evaluation of tissue-specific gene expression, markers for map-based cloning, and the annotation of genomic sequences. As of September 2009, Genbank contained 4.22 million entries of expressed sequence tags (ESTs) from seed plants. Although peanut is an important oil crop plants, the public available ESTs is only 92 thousand. The lack of sequence hampered peanut genomic research work. In this study, we constructed 5 peanut cDNA libraries, including one from *Ralstonia solanacearum* challenged roots, one from *R. solanacearum* challenged leaves, the remaining three are from normal growing roots, leaves and developing seeds respectively. Clustering and assembly of these ESTs resulted in a total of 14,547 unique sequences with 7,961 tentative consensus sequences and 6,586 singletons. We were able to identify putative function for 47.8% of these sequences. There were 212 sequences expressed throughout the libraries sampled, representing constitutively expressed sequences. 8874 sequences were uniquely expressed and were detected only in a single library. When challenged with *R. solanacearum*, differentially expressed genes from peanut leaves and roots were identified. According to these ESTs data, we were able to obtain genes with specific expression pattern. We were also able to identify a number of characterized as well as novel sequences that were unique to the compatible interaction between bacterial wilt pathogen and peanut, thereby providing a foundation for further understanding the mechanism of resistance and the molecular changes of peanut after pathogen challenge.

Screening of Peanut genotypes crossed at USA for leaf spot resistant and tested in field condition at Burkina Faso

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Variety Nama, a local peanut cultivar of Burkina Faso, type Virginia, 130-150 days cycle with small pods and having good resistant to early leaf spot and medium resistant to late leaf spot was been crossed with Texas varieties using biotechnology way. The hybrids generation F3 was evaluated in 2004 in Gampela/Burkina Faso. Hybrids generations F4 and F5 are evaluated respectively in 2005 and 2006. Annual selections allowed to obtained resistant material to leaf spot with note not more than 3 according to ICRISAT scale (1 to 9) and good yield. Twenty two performant varieties was been tested in the farmer field in 2007 and 2008 with the collaboration of farmer's Association (ATTRA/B) located at South-East of Burkina Faso in charge to transfer agronomy research technologies. These results obtained come from experimental station and farmer field condition. According to the result, some varieties perform well more than the local check ; some of them have obtained a good yield similar to the check with a good level of disease. This work continues with ATTRA/B in 2009 to confirm the results and try to identify one or more performant varieties resistant to leaf pot with good yield in the farmer field condition. The transfer of varieties to farmer field is our major objective to increase the peanut production in Burkina Faso and give some input to the farmer.

Development and evaluation of transgenic peanut for stress tolerance

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The interaction between drought stress in groundnut and the proline metabolism was observed in previous studies. Results obtained from trials in a stress facility suggested that the inability of the pods to maintain the activity of secondary metabolism under stress conditions resulted in colonization of pods by *A. flavus* and subsequent aflatoxin production. The proline levels in stressed plants were much lower than those in normal plants, while the glutamate level remained unchanged, giving an indication that proline metabolism was involved. It was therefore decided to incorporate a proline-enhancing gene into one of GCI's cultivars with the highest level of testa-resistance to test this interaction. A Δ^1 -pyrroline-5-carboxylate synthetase gene under regulation of a constitutive CaMV35S promoter was incorporated into a vector for transformation of the groundnut cultivar Akwa by means of particle bombardment of somatic embryos. Transgenic plants were regenerated successfully from single transformed cells through *in vitro* culturing methods. The developed transgenic lines were evaluated for drought tolerance and resistance to colonization by *Aspergillus flavus* in a stress facility at ARC-GCI, using standard pathology inoculation protocols. The reactions of the transgenic line on drought stress were monitored by evaluating relative water content (RWC) and CO₂ assimilation in leaves as well as yield. Proline as well as aflatoxin levels were determined. The results indicated that the transgenic line is not significantly more tolerant to drought stress than the control cultivars.

Assessing the cost effectiveness of pre- harvest options to Control Aflatoxin in Groundnut in West Africa: case of Mali

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The financial support from the Common Fund for Commodities (CFC) and INCO projects is highly appreciated. The authors are immensely thankful to groundnut growers, NGOs and government extension officials for their land offer as well as voluntary participation to organize the on-farm aflatoxin management trials in Kolokani and Kayes districts

Groundnut (*Arachis hypogaea* L.) is one of the major sources of rural livelihoods and foreign exchange earnings for many West Africa countries. It generates 40% and 42% rural cash earnings among groundnut producers in Mali and Niger, respectively (Ndjeunga et al., 2006). During the last 4 decades, West Africa lost its world groundnut production and export shares and competitiveness. Groundnut production shares declined from 23% to 15% whereas export shares decreased from 55% to 20%. To regain its competitiveness, groundnut productivity and production has to increase significantly, technologies to reduce aflatoxin contamination have to be promoted and grades and standards satisfied. For example, at EU standard of 0.5µg/kg, estimated profit losses for USA, China and Argentina is about US\$300 million.

Several approaches to minimize aflatoxin contamination in agricultural commodities have been developed and tested on-station (Waliyar et al., 2008, Craufurd et al., 2005). But there has been limited on-farm research to validate the efficacy of such practices in the semi-arid tropics under rainfed conditions. The effects of soil amendments with farm yard manure (FYM), lime (L), crop residues (CR) and their combinations were tested on farm to assess the level of aflatoxin contamination (Waliyar et al., 2005). In 2003, trials were organized in 8 farmers' fields in Kayes district and in 13 farmers' fields in both the Kolokani and Kayes districts during the 2004 crop season. The treatments included lime as source of calcium 400 kg/ha, FYM at 2.5 t/ha, cereal crop residues CR at 2.5 t/ha and their combination. A resistant (55-437) and susceptible (JL 24) cultivars were evaluated. Pod and haulm yields were measured. Aflatoxin was quantified using ELISA test.

The analysis of crop management options indicates that the set of non-dominated options were crop residues (CR), farm yard manure (FYM) and lime and crop residues (L+CR) in the resistant variety (55-437) and the susceptible check (JL 24). In effect, FYM+CR and L+CR are excluded from the optimal set. FYM+CR is dominated because for that level of investment it should have achieved a high level of aflatoxin reduction by being the optimal. The same reasoning applies to the application of lime and crop residues. Therefore, risk-averse decision makers would adopt crop residues, farmyard manure or crop residues with lime relative to the no-control measure where these management options are not used. Of the choices on the frontier

defined by non-dominated options, the choice will depend on risk and cost preferences of the decision maker.

This analysis would have been strengthened if it combined the output of the risk assessment with the output of the cost-benefit analysis enabling decisions makers to readily identify the risk reduction/cost trade-off curve associated with the risk reduction technologies so that they know ahead of time the potential impacts on social welfare in the event of an unexpected outcomes develops, or if an outcome has an unexpected consequences. The adoption of these technologies will largely depend on the accessibility and availability of these inputs to farmers. In addition, given the costs, uptake of any option will increase if farmers have access to credit facilities. The government's role at creating a conducive environment to facilitate access to inputs including credit is a prerequisite.

Evaluation of five peanut (*Arachis hypogaea* L.) genotypes to determine plant response to mid- and late-season drought

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Drought is an abiotic factor that causes yield loss in peanuts. An understanding of molecular plant response to water limitation is critical to the development of new varieties that can withstand short or long term drought stress. Five peanut genotypes were chosen based on various degrees of drought responses and were subjected to deficit irrigation for mid-season (40 – 60 DAP) and late-season (90 DAP to harvest) using rainout shelters in the field. Leaf tissues were collected at different times during the stress periods. Measurements of relative water content (RWC) and soil moisture were taken to correlate plant water stress. Harvest Index for each genotype will also be measured and correlated to the level of plant stress. Yield, grade, and aflatoxin contamination data will also be collected. The initial goal of this study is to correlate drought stress levels of the five genotypes using these agronomic traits. Gene expression studies will follow using leaf, root, and seed tissues to verify and further characterize important biochemical pathways in peanut drought response.

Heritability Estimates for drought resistance related traits in cultivated peanut (*Arachis hypogaea* L.)

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Drought is a major factor in reduced productivity in peanuts. A good knowledge of the inheritance of SLA, $\delta^{13}\text{C}$, and HI may facilitate selection for drought resistant cultivars in peanut breeding programs. The objectives of this study were to estimate the heritability of SLA, $\delta^{13}\text{C}$, and HI traits in peanuts and investigate the relationships among these traits. Fifteen genotypes were selected to measure the heritability of these traits using the variance component method based on an entry-mean basis. These 15 genotypes were planted in a randomized complete block design with three replications in drought resistance 2007 and 2008 at Headland, Alabama and Dawson, Georgia with and without irrigation. The leaf samples were taken at the 85th day after planting for measurements of SLA, and $\delta^{13}\text{C}$. The HI was calculated on mature plants at 135 days after planting. Analysis of variance (ANOVA) showed that highly significant differences were found for year, location, genotype, and genotype x location for SLA, $\delta^{13}\text{C}$ and HI traits ($p=0.01$). The results from ANOVA demonstrated that the heritability for SLA, $\delta^{13}\text{C}$ and HI was 0.32, 0.90, and 0.61, respectively. $\delta^{13}\text{C}$ was negatively correlated with SLA, HI, and Yield. HI had a stronger association with pod yield. However, under drought condition $\delta^{13}\text{C}$ had the highest correlation coefficient with yield. This implies that the selection for HI would result in a greater response to drought resistance and yield than the selection for SLA in breeding programs. $\delta^{13}\text{C}$ can be used to discriminate the degree of drought resistance in peanuts under stress condition.

Early stages of fungi interaction with wild and cultivated of *Arachis* by scanning electron microscopy

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Fungi diseases such as late leaf spot caused by *Cercosporidium personatum* and rust caused by *Puccinia arachidis* are important biotic stress factors that can affect peanut (*Arachis hypogaea* L.) production. Nevertheless, resistance is found in different *Arachis* wild species. Aiming the better understanding of *Arachis* resistance mechanisms, ultrastructural analysis of the early stages of the interaction were here carried out. To determine key steps of plant pathogen interaction, detached leaves of *A. stenosperma* V10309 (wild, resistant) and *A. hypogaea* cv. IAC-Tatu (cultivated, susceptible) were inoculated with *C. personatum* or *P. arachidis* and collected between 3-96 HAI (Hours After Inoculation). Samples were processed for scanning electron microscopy and observed in SEM Zeiss DSM 962. Adhesion and germination of *C. personatum* was detected 6HAI in both species, although hyphae growth and distribution was larger in cv. Tatu. Penetration of the hyphae in the stomata opening was observed 72HAI only in Tatu. In both species, adhesion of *P. arachidis* spores was observed 3HAI, germination 6HAI and the development of appressoria, 12HAI. Hyphae proliferation was observed 24-48HAI in both species, but more evident in cv. Tatu. Hyphae presence was observed in the stomata 72HAI only in Tatu. Current analyses demonstrated that infection in *A. stenosperma* is mostly controlled during later steps of interaction (proliferation and penetration of the hyphae) and not during spore adhesion, germination or appressorium formation. These data will support further molecular studies during sampling to facilitate the isolation of genes associated with fungi resistance.

Functional genomics and resistance gene discovery in *Arachis*

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Functional genomic resources for a number of wild *Arachis* species (ESTs) were developed as a basic resource for gene discovery, interpretation of genomic sequences and marker development. This data will contribute for the analysis of the complexities of gene expression patterns and functions of transcripts in *Arachis*. Using an integrated approach associating morphological, physiological and transcriptome data, wild species of *Arachis* have been characterized in order to identify species harboring nematode (*Meloidogyne* spp.) and foliar fungi (leaf spots) resistances. A total of 13 candidate genes have been identified in *A. stenosperma* ESTs. In some of these sequences, well characterized SNPs have been identified, using the parentals of the AA genome mapping population, and later converted into genetic markers. Candidate resistance genes are being further characterized through *in situ* hybridization, qRT-PCR and microarray assays in order to evaluate their spatial, temporal and expression profiles and potential role in the mechanisms of plant response to these diseases.

Aflatoxin occurrence and distribution in Malawi

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The International Crops Research Institute for the Semi-arid Tropics (ICRISAT) in collaboration with the National Smallholder Farmers Association of Malawi (NASFAM) are implementing a project to map the occurrence, significance and distribution of Aflatoxin contamination in Malawi. The study covers major groundnuts producing districts: Lilongwe, Mchinji, Kasungu, Mzimba but also in high altitude areas of Phalombe and Ntchisi and the low lying areas of Salima, Nkhotakota and Chikwawa. Sample collection was undertaken from February – March, 2009 targeting samples harvested from the 2007/08 season and stored for 8 – 11 months under different conditions as is the common practice in Malawi. A total of 696 samples of groundnuts and maize inclusive of grains and processed foods were collected from farmers households, local market vendors, shops, supermarkets and warehouses. For each sample, passport data inclusive of GPS coordinates were captured to facilitate development of GIS based risk map. Samples were prepared for analysis using ELISA procedure and results compared with known standards. Aflatoxin contamination in samples ranged from 0.0 ppb to as high as 2197ppb. Groundnut powder had the highest proportion of highly contaminated samples - 73% registering levels above the EU acceptance. Approximately 25% of all market samples of powdered groundnut had contamination levels above 100ppb. For groundnuts, 43% of all samples in farmers households, 49% from local markets, 58 – 60% from shops and supermarkets, and 41% from warehouses, had aflatoxin levels above the EU safe limit. District wise, the high rainfall districts registered some of the highest contamination (Mchinji 0.0 – 2196ppb), 43% of which would be rejected under EU standards and Mulanje (6.6 – 554ppb) 100% of which would be rejected under EU standards. The drought prone districts Machinga, Salima, Nkhotakhota also registered high proportions of contamination (44 – 86% of samples). Mzimba registered the highest quality nuts (0.0 – 12.5 ppb) with 82% meeting or exceeding the EU safety limits. Similarly for maize, 29% of samples in farmers households, and 14% in the local markets exceeded the EU safe limits. The study findings reveal that, in general, there was less contamination in maize than in groundnut samples from similar sources. This study has implications for planning an integrated approach for management of aflatoxin contamination.

Test en milieu paysan de greniers traditionnels améliorés pour la Conservation de l'arachide dans la Région de Kayes

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Trials have been conducted mainly in area where groundnut production is intensive in Mali. The main objectives of the present study were to evaluate the performance of two models of improved traditional granaries developed during the realization of the project named "Implementation of methodology to struggle against food contamination by *Aspergillus flavus*" and "Inco Aflatoxin" project.

The evaluation has led to reduce *Aspergillus flavus* quantities averagely: 1.2×10^3 / 100g for two types of improved traditional granaries against an average of 24×10^3 / 100g for usual traditional granary, which correspond to a reduction of 95%. During the trials a maximum rate of 56 ppb of Aflatoxin B₁ was found in classical traditional granaries. While, the Aflatoxin B₁ content was about 15 ppb in the improved traditional granaries corresponding to a reduction of 73%. Determining the number of parasites inhabiting has shown the same trend with a maximum value 16 (weevils + bruchid) in the classical traditional granaries against 3 in both improved traditional granaries.

Based on the obtained results, training has been provided to producers on the construction techniques and on the efficient operation of improved granaries.

Potential assessment for recombining resistance to aflatoxin production in groundnut (*Arachis hypogaea* L.) and related resistance mechanism

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Integrating high oil content and resistance to aflatoxin production would be a key objective in further breeding for groundnut (*Arachis hypogaea* L.) in developing countries where the crop is mostly used for oil with aflatoxin contamination being a great health concern. Through artificial inoculation with *Aspergillus flavus*, aflatoxins were quantitatively determined in 117 groundnut recombinant inbred lines (RILs) derived from “Yuanza 9102 × Zhonghua 5”. Significant variation of aflatoxin content was observed among the RILs and several recombinants with reduced AFT were identified, indicating that there was a feasible potential for improving the resistance to AFT formation by accelerating the concerned minor effect genes or locus. This conclusion was also verified in another group of advanced lines derived from a cross of “K01-6 × Kainong-30”. There was no significant correlation between the resistance to AFT formation and pod mass, oil content, protein content, oleic and linoleic content, and resistance to bacterial wilt (BW), indicating that there is a potential for integrating resistance to AFT production with other important characters. Several elite RILs with large pod, high oil content and desirable resistance to both BW and AFT production were identified. The activity of peroxidases (POD), polyphenoloxidase (PPO), phenylalanine ammonia-lyase (PAL) and contents of lignin and chlorogenic acid in selected groundnut genotypes with different resistance to AFT production were determined after inoculation with *A. flavus*. The results indicated that the activity of all the enzymes involved in resistant genotypes increased much rapidly than that of susceptible ones. It could be concluded that the mechanism of AFT resistance in the recombined lines was similar to Zhonghua 6 (resistant control).

Exploring the scope of Cost-Effective Aflatoxin Risk Reduction Strategies in Maize and Groundnut Value Chains so as to Improve Market Access of the Poor in Africa

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Aflatoxin is a toxic, carcinogenic by-product of fungi that colonize maize and groundnuts, among other crops. In Sub-Saharan Africa, maize is significant as both a livestock feed and as a staple accounting for 42 percent of the cereal crop. Groundnuts are an important cash crop controlled largely by women. More than 4.5 billion people in developing countries may be chronically exposed to aflatoxins in their diets. Common to tropical climates, aflatoxin contamination most often occurs when crops suffer stress, such as drought or insect infestation. Aflatoxins are considered unavoidable contaminants of food and feed, even where good manufacturing practices have been followed. While developed countries regularly test for aflatoxin, many developing countries lack cost-effective ways to test and many smallholder farmers lack ways to prevent contamination, which ultimately impedes their ability to market crops.

Recently the International Food Policy Research Institute (IFPRI), International Maize and Wheat Improvement Center (CIMMYT), International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), University of Pittsburgh, Uniformed Services University of the Health Sciences, Kenya Agricultural Research Institute, Institut d'Economie Rurale (Mali), ACIDI/VOCA, and the East African Grains Council have joined forces with funding from the Gates Foundation to analyze the impact of aflatoxin contamination on the livelihoods and health of people in Kenya and Mali. The project is called Aflacontrol: Improving lives in Africa.

The project will seek to map areas at highest risk, identify cost-effective control measures to reduce exposure to aflatoxins, and disseminate findings to key stakeholders and policy makers. The project will also assess knowledge, attitudes, and practices held by farmers, consumers, and others involved in agriculture regarding aflatoxin, as well as their willingness to pay for testing and use of control strategies. A database of aflatoxin prevalence in selected sites in Kenya and Mali will be created to measure the effectiveness of control strategies. Risk maps that identify high risk areas for aflatoxin contamination will also be developed. This talk will highlight the objectives and planned outputs for the project.

Tagging and Mapping of Nutritional Quality in Groundnut

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Groundnut, being the 3rd most important source of edible oil and 4th most important source of vegetable protein, deserves proper attention by the breeders for genetic enhancement of nutritional quality. In this regard, protein and oil content and oil quality as indicated by the ratio of oleic to linoleic acid are the most important traits. But laborious and time consuming process in estimation of these traits has made it difficult and uneconomical to screen large segregating populations resulting in limited progress with conventional approaches. In this scenario molecular marker assisted breeding could be a justified option for enhancement of quality traits. In a study consisting of 146 RILs obtained by crossing widely differing genotypes *viz.*, GPBD 4 and TG 26, QTLs were identified by employing 53 polymorphic microsatellite markers. A total of 6 QTLs (1.5 to 10.7% R²) for protein content, 4 QTLs for oil content (1.5 to 9.1% R²), 4 common QTLs for oleic (0.6 to 9.7%) and linoleic acids (0.7 to 9.0% R²) and two QTLs for O/L ratio (1.0 to 6.8% R²) were identified. A major QTL identified for rust resistance (XIP103) had substantial contribution for oil content (7.9 to 9.1 % R²). One QTL (TC6H03) showed significant contribution for protein (10.7 %), oleic acid (9.7% R²) and linoleic acid (9.0% R²). The identified QTLs are being validated for use in marker assisted crop improvement.

Identification and evaluation of wild *Arachis* species with high oil content

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Wild *Arachis* species are important resource for genetic improvement of the cultivated peanut (*Arachis hypogaea* L.). In the present study, oil content in 87 wild *Arachis* accessions was tested. There were many accessions with high oil content. The minimum, maximum and average oil contents in the wild *Arachis* genotypes were higher than the corresponding contents in the cultivated germplasm. Twelve accessions with high oil content (more than 58%) were identified. The percentage of genotypes with high oil content was 13.79% which was higher than the corresponding percentage in cultivated peanut resource. *A. appressipila* had the highest oil content as 62.90%. The relationship and genetic diversity of the 12 high oil *Arachis* accessions and several cultivated lines were investigated based on SSR markers. The diploid species *A. villosa* of section *Arachis* was closely related to the cultivated peanut. The DNA fingerprints of 12 accessions with high oil were constructed based on SSR markers.

Cloning and expression analysis of genes encoding ACCase enzyme subunits from *Arachis hypogaea*

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Peanut (*Arachis hypogaea* L.) is an important oil crop and widely grown in the world. It is of great importance to study the regulation and fatty acid biosynthesis pathway in the view of improving oil quality and increase oil content through biotechnology based approaches. ACCase is the enzyme that catalyzes the formation of malonyl-CoA from acetyl-CoA and bicarbonate in the first committed step of de novo fatty acid synthesis. Two structurally distinct forms of ACCase have been identified in higher plants, the multi-subunit (MS) and the multifunctional (MF) ACCases. In this study, candidate genes of subunit BCCP, BC, α -CT, β -CT and MF ACCase gene were isolated from peanut by sequencing a full-length cDNA library and homology based cloning. Primary structures of peanut ACCase are highly conserved, especially the biochemical function domains, compared with other plants and *E. coli*. The MF ACCase of peanut and *Arabidopsis* shared conservative structure of genomic organization. RT-PCR analysis showed ACCases were expressed in all detected peanut tissues with a varied expression level. In peanut seed development, BC, α -CT, β -CT and MF ACCase mRNAs were expressed highly at 60 DAF. These results will provide a basis for investigations of the roles of ACCases genes in oil deposition and other physiological processes in peanut.

Manipulation of the toxigenicity of *Aspergillus flavus* soil populations to control aflatoxin contamination in peanuts.

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Aflatoxin contamination of peanuts, which results from invasion and growth of the fungi *Aspergillus flavus* and *A. parasiticus*, not only threatens human and animal health, but it also causes great economic loss to peanut industries in most peanut-producing countries. Research in recent years has shown that biological control of aflatoxin contamination of peanuts can be achieved by applying a competitive, non-aflatoxigenic strain of *A. flavus* to soil during the middle of the growing season. A formulation for establishing that strain in the field was developed and commercialized under the trade name, AflaGuard®. Large-scale studies were conducted to determine the efficacy of the biopesticide for control of aflatoxin in peanuts grown in the southeastern USA. AflaGuard was applied mid-season to approximately 2000 ha of peanuts at a rate of 22.5 kg/ha. Dilution plating of soil samples taken from representative fields of treated and untreated peanuts showed that the incidence of toxigenic strains of *A. flavus* was reduced from 71.1% in untreated fields to 4.0% in treated fields. Analyses of farmers' stock peanuts from treated (n = 404) and untreated (n = 178) fields showed that mean aflatoxin in treated peanuts (11.7 ppb) was reduced by 85.2% compared with untreated peanuts (78.9 ppb). At two locations where treated and untreated peanuts were stored under identical conditions, analyses of shelled lots showed that AflaGuard reduced aflatoxin in edible grade peanuts by 69.4 and 97.5%, respectively. At both locations, no treated, shelled lots were rejected for human consumption because aflatoxin concentrations exceeded allowable levels.