5th International Conference of the Peanut Research Community

on

Advances in *Arachis* through Genomics and Biotechnology

(AAGB-2011)

Brasilia, Brazil, 13th -15th June 2011

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Welcome to Brasília

After visiting all other continents, the 5th International Conference of the Peanut Research Community is being held in South America, the origin of cultivated peanut.

The aim of this AAGB meeting is to bring together scientists involved in all aspects of peanut research and technology, both pure and applied. Whilst maintaining a broad focus, the meeting has a special emphasis on promoting exchange of ideas within, and at the interfaces of, modern genetics, genomics and biotechnology.

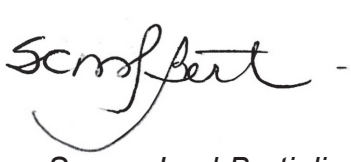
Over the last few years we have seen great developments in the genetic and genomic resources for peanut. These advances, together with new sequencing technologies are turning the prospect of sequencing the genome of peanut into a reality. The developments hold great prospects for more efficient research and greater understanding of peanut biology.

Presentations will be divided into five Sessions: Allelic Diversity and Germplasm Resources, Crop improvement, Product Quality and Safety, Genetic Mapping and Gene Discovery, Genome Structure and Genome sequencing. In these sessions we have the privilege of hosting eminent scientists from all over the World. Numerous poster presentations will also be made. This allows the presentation of work in a detail that is not possible in oral presentations and ensures the full participation of students. We will also discuss the Peanut Research Strategic Plan for the next Five years in the parallel sessions. Overall we aim to cover a good cross section of subjects, in an informal atmosphere that stimulates discussions, and the formation of new collaborations and friendships.

For logistics, the AAGB has had special support from the host institute, Embrapa Genetic Resources and Biotechnology: heads of departments, colleagues and support staff have all played their parts with enthusiasm and energy. Many thanks are due to them all! Many thanks are also due to our sponsors who were so generous and made this event possible.

Lets all have a fruitful and enjoyable time!

David Bertioli



Soraya Leal-Bertioli

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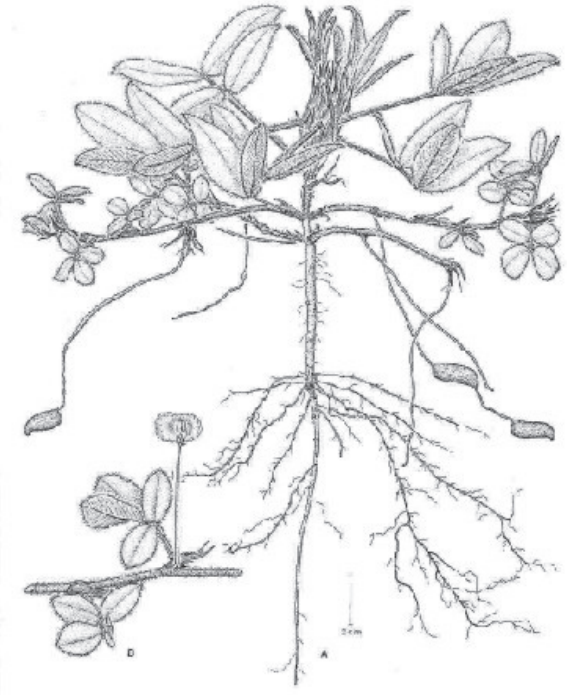

Key Note presentation

Notes

Feeding the hungry world with peanuts

H Valentine[1]*.

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Each year 5.1 million children under the age of 5 die from malnutrition. A new product is beginning to be used to reverse that terrible statistic, a peanut based therapeutic food. In my presentation I will outline the breadth of the problem of malnourishment and show how the International Peanut Genomic Initiative is an integral part of the solution. This presentation will contain 4 parts: (1) where is the malnourishment and why do we need a better peanut; (2) what characteristics do these new peanuts need to have; (3) what else is required in addition to these new characteristics; (4) how will the product use be expanded. The international peanut genomics group of researchers has the tools to make these concepts a reality.

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Notes

Current status of peanut breeding in São Paulo, Brazil, and surrounding states

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Peanut production in Brazil is highly concentrated in the state of São Paulo and neighboring states, comprising around 100,000 hectares, which represents 80% of the planted area in the country. In nearly 70% of this region, peanuts are planted in rented areas, in rotation with sugarcane. Breeding is currently focused in developing cultivars of runner growing habit and kernels of the runner commercial group, to attend both internal and exporting markets. The main breeding objectives are those related with a technified system of production, that is: high yield, shelling quality, kernel size distribution to meet exporting standards and high oleic content. Breeding program at Instituto Agronômico (IAC) is also working on more specific objectives. One is to select for runner growing habit with a cycle duration not longer than 120 days. This time period, from planting to harvesting, is a requirement to better fit the peanut crop in the sugarcane rotation system. Other objectives are to develop cultivars with high levels of resistance to foliar diseases, and to search for resistance to thrips and redneck worm, the key pests of peanuts in São Paulo.

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Strategic advances in peanut genomics research: Establishing research plans and expectations for 2012 to 2016

Notes

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Successful research Initiatives follow a three step process that helps keep their activities in the spotlight with stakeholders & investors. It begins with forums for communication such as AAGB meetings. Next, a solid strategic foundation is needed for implementing research that addresses stakeholder needs. Then, return-on-investment is demonstrated by impact of accomplishments that are relevant to objectives in the strategic plan. This process affords program transparency and accountability; attributes that have helped launch the Peanut Genome Project. As we prepare to sequence the Arachis genome, it is timely to draft the next strategic plan for peanut genomics research. The scope of the new plan will not change. However, the components of the new plan will follow the session topics for AAGB-2011. Facilitated breakout sessions will be convened this afternoon to consider these topics in more detail and capture your input. Each group will be asked to choose one or more Goals that define a desired outcome from research under a component of the plan. Goals may have one or more Objectives (Performance Measures). The Objective's title should define an area of work or problem that needs to be solved. Each Objective should include: a statement indicating why the objective is needed; one or more statements of the research approach or what will be done; and a list of anticipated products (deliverables or milestones) over the next five years. Each group will present a summary report in General session. The writing team will post progress reports on our website, www.peanutbioscience.com.

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Notes

The genome of *Arachis hypogaea*: genetic linkage map will aid the whole genome sequence assembly

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The allotetraploid peanut genome assembly will be a valuable resource to researchers studying other polyploidy species, in addition to providing new insight into peanut genome evolution and domestication other than facilitating quantitative trait locus (QTL) analysis and the tools for marker-assisted breeding. Unlike other plant genome sequencing projects there is no complete linkage or physical map already available at this time. Therefore, a peanut genome-wide linkage map will aid genome assembly, acting as an independent resource against which contig assembly can be validated. The objective of this study was to develop a comparative integrated map from two recombinant inbred line (RIL) populations with diverse backgrounds in which two runner type cultivars, one Spanish type cultivar and one breeding line derived from a cross of Virginia type and *hirsuta* type were used as parents. A total of 4576 simple sequence repeat (SSR) markers from three sources: published SSR markers, newly developed SSR markers from expressed sequence tags (EST) and from bacterial artificial chromosome (BAC) end-sequences were used for screening polymorphisms. Two CAPS (cleaved amplified polymorphic sequence) markers were also included to differentiate *ahFAD2A* alleles and *ahFAD2B* alleles. A total of 324 markers were anchored on this integrated map covering 1,352.1 cM with 21 linkage groups (LGs). Combining information from duplicated loci between LGs and comparing with published diploid maps, 7 homoeologous groups were defined and 17 LGs (A1 to A10, B1 to B4, B7, B8, and B9) were aligned to corresponding A-subgenome or B-subgenome of diploid progenitors. One reciprocal translocation was confirmed in the tetraploid cultivated peanut genome. Several chromosomal rearrangements were observed. This genetic linkage map and others could provide a framework for QTL analysis and a scaffold for integration of the physical map and genome sequence assembly.

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Challenges to the Future of Peanuts: A Genomic Solution?

Notes

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Peanut, also known as ground nut is second only to soybean in global economic importance among the food. Worldwide, 36.5 million metric tons of peanuts were produced on 25.2 million hectares in 2009. It is a major source of cooking oil for the most populous countries such as China and India. The nutrient composition of peanut makes it a good source of nutrition for many developing countries. For this reason peanut is now utilized as a source of nourishment for malnourished children around the world. The use of Plumpy nut, Mamba and other brands by relief agencies is a testimony to this fact. It is estimated that peanut contributes over $65 billion dollars to the global economy. The competitiveness of peanut producers in various domestic and global markets is threatened by losses in productivity and quality that are attributed to diseases, pests, environmental stresses and allergy or food safety issues. Competing crops such cotton, soybean and corn have seen tremendous improvements in yield, disease resistance, and quality. Hence, are taking up more available acreage because they require less input cost, and therefore more profitable for growers. The objective of the presentation is to share relevant information on quality, compositional and nutritional information that highlights the need for peanut improvement via the understanding of the genetic make up of the crop. Additionally, the intent is to challenge all researchers to focus on improving the competitiveness, nutritional and quality of the crop.

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Notes

Apply Next Generation Sequencing into Peanut Research

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Peanut is the fourth most important oilseed crop in the world. However, the genetic and breeding research in peanut is far behind many other crops. With the development of sequencing technology and bioinformatics, it is possible to construct the peanut genome map and build the platform for peanut research. We have developed a novel method for *de novo* assembly of large genomes from short-read sequences and successfully assembled the cucumber, Chinese cabbage and potato genomes. Many of the genes related to development or resistance in these species have been annotated, which have resulted in applications for improving breeding strategies and better varieties. And we also apply deep sequencing technologies into biology research, such as resequencing, transcriptome sequencing, digital gene expression tag profiling, small RNAs and degradome sequencing etc. These next generation sequencing technologies would be very useful for germplasm analysis, molecular marker development, diversity studies, qualitative and quantitative trait mapping, linkage mapping, gene expression profiles, small RNA identification, the interaction of miRNA and mRNA etc.

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Employing Microsatellite and SNP Markers to Track Functional Mutations and Evaluate Genetic Diversity in the USDA *Arachis* Germplasm Collection

Notes

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Peanuts (*Arachis hypogaea* L.) are nutritious because their seeds typically contain high amounts of oil, protein and other phytochemicals such as folic acid, tocopherol, and antioxidants; therefore, they are an important oil seed crop worldwide. The USDA Plant Genetic Resources Conservation Unit maintains over 10,000 *Arachis* accessions including both cultivated and wild germplasm. A core (831 accessions) and mini core (112 accessions) collection were established to represent maximum genetic diversity with minimal redundancy to help researchers rapidly mine important traits from a manageable sample set. The mini core, botanical varieties, and some wild relatives have been evaluated for allelic variation with microsatellite and SNP markers. The SNP markers were developed to detect wild type and mutant alleles in both *ahFAD2A* and *ahFAD2B,* which are known to affect oleic acid (C18:1) and linoleic acid (C18:2) accumulation. These *ahFAD2* SNP markers were an effective tool utilized in a breeding program to select high oleic material in each generation and employed to quantify all *ahFAD2* genotypes and their resulting phenotypes. Further, biochemical data such as total oil content, fatty acid composition, flavonoids, resveratrol, and morphological traits were also collected from the mini core accessions. The molecular markers and phenotypic trait data were employed to assess allelic variation, phylogenetic relationships, population structure, and association analysis of the mini core accessions. Genetic variation was revealed in the mini core and a few markers associated with phenotypic traits were identified. The information obtained will be useful for enhancing breeding efficiency and improving seed quality in peanuts.

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Notes

Developing Introgression Pathways for Gene Transfer to *Arachis hypogaea* L.

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The effort to develop introgression pathways began at Texas A&M University in 1973 with the cross *A. diogoi* X *A. cardenasii* to combine early and late leafspot resistance for transferring to *A. hypogaea*. This partially fertile hybrid [52% pollen stained (ps)] was later crossed with *A. batizocoi,* the only known B genome species in 1978. The tri-species hybrid (0.01% ps) was doubled to 40 chromosomes. The subsequent cross with *A. hypogaea* was successful, producing progeny with 92% ps. A backcrossing program was then initiated with *A. hypogaea* cv. Florunner as the recurrent parent. From this pathway resistance to leafspot – early and late, rootknot nematode and peanut rust have been transferred into cultivated peanut. Three nematode resistant cultivars have been released from this source and leafspot resistance is approaching release. NemaTAM has partial resistance to rust. Molecular markers are now playing a key role in the nematode resistance program and it is hoped that the same will be true for leafspot and other traits in the future. Other pathways for introgression being researched involve A and B genome species to transfer southern blight resistance from *A. stenosperma*. A reliable screening technique and molecular markers will facilitate this transfer. Another pathway involves several accessions and species of the section *Erectoides* using the species *A. vallsii* as a bridge between sections *Arachis* and *Erectoides*. *Arachis vallsii* was originally classified as section *Procumbentes*. However, research in Brazil and the USA has shown that *A. vallsii* should be classified with *Arachis* section materials.

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Molecular Variability Among *Arachis* Species

Notes

HT Stalker[1]*, SP Tallury[1], KW Hilu[2], ED Jones[1], ME Mariano[1] & S Knapp[3].

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Molecular markers are useful tools for maintaining germplasm purity, hybrid identification, trait selection, and understanding relationships among taxa. In peanut, a large amount of molecular variation has been observed among and within *Arachis* species. In a study of 42 *Arachis* species using 50 simple sequence repeat (SSR) markers, individual species greatly varied in the number of bands with only 17 in *A. pusilla* to 134 in *A. correntina*. Between one and 12 unique bands were identified in 30 species. The unique banding patterns observed should be useful to confirm species identities, help maintain species purity, and confirm interspecific hybrids in crossing programs. To test the systematic treatment of the genus, nuclear ITS and plastid *trn*T *trn*F sequences from 48 species were analyzed. ITS cloning of *A. hypogaea* indicated the presence of A and B genome alleles and chimeric sequences. In a cluster analysis, species from section *Extranervosae* emerged as the first lineage in the genus, followed by sections *Triseminatae* and *Caulorrhizae*. *Arachis burkartii* formed a separate group whereas members of sections *Erectoides*, *Procumbentes*, *Trierectoides*, *Heteranthae*, and 4x *Rhizomatosae* clustered together. One of the two large terminal lineages included the B and D genome species and aneuploids, while a second terminal lineage included the A genome species. The results substantiated the sectional treatment of *Caulorrhizae* and *Triseminatae*, but also indicated that sections *Arachis, Erectoides, Procumbentes,* and *Trierectoides* are not monophyletic. A detailed study of the genus *Arachis* with denser taxon sampling, additional genomic regions, plus information from morphology and cytogenetics is needed for a comprehensive assessment of the genus *Arachis*.

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Notes

QTL analysis of early leaf spot resistance and agronomic traits in an introgression population of peanut

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Early (*Cercospora arachidicola* S. Hori) and late (*Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton) leaf spot are serious pathogens in many peanut growing countries, and can cause significant defoliation and reduction in yield. Wild species of peanut contain many useful alleles, especially for resistance to diseases and pests. However, there are significant differences between wild and the cultivated peanut species for many agronomic traits, and these can hinder the incorporation of useful alleles from wild species. We have generated an advanced backcross population from a cross between the cultivar Florunner and TxAG-6, where TxAG-6 is a synthetic diploid derived from the cross [*A. batizocoi* x (*A. diogoi* x *A. cardenasii*)]^{4x}. Plants of the BC_3F_1 generation were screened in the greenhouse after inoculation with early leaf spot. Five QTLs were found on four linkage groups for reaction to leafspot infection. In addition, BC_3F_2 plants were screened in the field for agronomic traits. A total of 36 QTLs were identified by composite interval mapping. Of these, 7 were for number of lateral branches, 6 for lateral branch length, 2 for main stem length, 9 for seed length, and 12 for seed width. Co-mapping of QTLs suggested that several QTLs may have been for closely-linked QTLs or for genes with pleiotropic effects. Identification of markers for domestication is expected to be useful in marker-assisted breeding efforts.

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Genetic mapping of cultivated peanut with genomic SSR and transposon markers screened by *in silico* polymorphic analysis

Notes

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Because of its extremely low polymorphism, a high-density linkage map in cultivated peanut has not been reported yet. We previously developed 2189 DNA markers including genomic SSR, EST-SSR and transposon-based markers, and then obtained 283 (13%) polymorphic markers between mapping parents of cultivated peanut by experimental polymorphic analysis. In order to improve efficiency of polymorphic marker identification, we performed *in silico* analysis for pre-screening of candidate polymorphic markers before experimental screening. A total of 41679 sequences was obtained from genomic SSR- and transposon-enriched libraries of the two peanut varieties using the ABI-3730xl sequencer, and was used for identification of polymorphic SSR or transposon-insertion/deletion regions by comparison of corresponding sequences of the two varieties. As a result, a total of 2367 candidate polymorphic markers were identified from 5246 SSR or transposon insertion regions, and 47% of the 2367 were confirmed presence of polymorphisms by a capillary fragment analyzer. We have performed segregation analysis of two F_2 mapping populations with a total of 1386 markers, i.e., the previous 283 and the present 1103 markers, for high-density linkage map constructions. With the obtained information, peanut breeding program for improvement of oleic acid content has been started through marker-assisted selection.

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Financial support: Chiba prefecture and MEXT, Japan

Notes

Advances on the scientific knowledge and use of peanut wild relatives in Brazil

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Since the description of *Arachis* by Linnaeus, and the first collection of a wild *Arachis* species in 1819, in Central Brazil, substantial scientific knowledge has been accumulated on the genus, though mostly concentrated on *A. hypogaea*. Of 80 *Arachis* species so far described, 62 are native to Brazil, where the non-native peanut is a widespread crop. Species producing high quality forage found their way into the seed market, and a few additional species are still cultivated for grain in remote indian communities. Only four Brazilian botanists authored descriptions of Brazilian species, but the impact of local biosystematic research in the last three decades is widely recognized. Strong cooperative ties with national and international research institutions and fraternal collaboration with foreign peanut specialists promoted Brazilian participation, and eventually the leadership, of international initiatives, improving local scientific outputs, and strengthening the infrastructure for advanced research. Broad collection efforts commanded from the geographic center of occurrence, with maintenance of the acquired germplasm in a very diverse genebank, was the starting point, followed by characterization of accessions using increasingly accurate cytological, genetic and biochemical methodology. Evaluation of forage production and phytopathological attributes helped to grant institutional support for continuing germplasm rescue and investigation. Intraspecific crosses helped to realize taxonomic relationships and to reconstruct the events related to the origin of the peanut. Human resources development has been a landmark, in a framework of scientific cooperation towards the sustainable use of the genetic resources of *Arachis*, unfortunately nowadays hindered by regulations restrictive to international exchange.

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Financial support: Embrapa & CNPq

Markers, Maps and Molecular Breeding in Cultivated Groundnut: Not a Dream Anymore!

Notes

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Groundnut (*Arachis hypogaea* L.) is the fourth largest oilseed crop in the world and grown extensively throughout the semi-arid tropics of Asia, Africa and Latin America. Its genetic yield potential has been adversely affected due to several biotic and abiotic stresses. Marker-assisted selection offers an important tool to enhance tolerance/resistance to above stresses which are difficult through conventional breeding. However, a very limited amount of genomic resources were available until recently. As a collaborative effort with several partners, large scale genomic resources such as simple sequence repeat (SSR) and Diversity Array Technology (DArT) markers have been developed recently. For instance, 4,245 SSR markers assembled from public domain or collaborators were screened on parental genotypes of five mapping populations (TAG 24 × ICGV 86031, TAG 24 × GPBD 4, TG 26 × GPBD 4, ICGS 44 × ICGS 76 and ICGS 76 × CSMG 84-1) segregating for drought tolerance and foliar diseases namely late leaf spot (LLS) and leaf rust (LR). In general, 6-10% polymorphism was observed with the SSR markers tested. Genotyping of polymorphic markers has facilitated development of five genetic maps with moderate marker density (83-191 SSR loci per cross) for respective mapping populations. Based on these genetic maps, a consensus genetic map comprising of 362 SSR loci spanning 3,543 cM was constructed. In parallel, mapping populations were phenotyped extensively for drought tolerance traits, LLS and LR. Detailed QTL analyses provided 153 main effect QTLs and 25 epistatic QTLs for drought tolerance traits. On the other hand 19 QTLs each for LLS and LR were observed. One major QTL for LR contributing >50% phenotypic variation is being introgressed in three elite groundnut varieties namely ICGV 91114, JL 24 and TAG 24 by using marker-assisted backcrossing approach. In summary, molecular markers, genetic maps and QTLs are ready for facilitating molecular breeding activities to improve crop productivity of cultivated groundnut in marginal environments of Asia and Africa.

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Notes

Mining for induced and natural variation in peanut genes

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Peanut, *Arachis hypogaea* L., has limited genetic diversity within the cultivated gene pool, and relatively little is known about the functions of specific genes. Induced genetic variation could expand our knowledge of gene function as well as generate variants useful for genetic improvement of the crop. Access to natural variation found in wild relatives of peanut also can be achieved when species are cross compatible, for example through synthetic tetraploid introgression. TILLING (Targeting Induced Local Lesions IN Genomes) and EcoTILLING are reverse genetics approaches to detect induced or spontaneous mutations, respectively, at the DNA level when no visible phenotype may be displayed. We have used TILLING and EcoTILLING to identify allelic variation in two allergens, Ara h 1 and Ara h 2. Allergen genes have been targeted initially because peanut allergy affects ~1% of the US population and evidence suggests that incidence is increasing in the developing world. Several seed storage proteins, including Ara h 1 and Ara h 2, are allergenic although their primary function in plants is nutrient reservoir activity. One knockout mutation has been recovered in one copy of each of these genes along with a spectrum of missense alleles. The potential, therefore, exists to modify allergen composition or content in cultivated peanut and thereby reduce allergenicity.

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Identifying and exploiting biodiversity in wild relatives of crops

Notes

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Crop improvement relies on the exploitation of new biodiversity and new combinations of diversity. We will discuss our work on genome structure and evolution that underpins the measurement and exploitation of diversity in crops and their wild relatives. Polyploid crops enable new genome combinations to be made, and the transfer of chromosomes carrying useful agronomic traits. Examples of our work from wheat, banana, crocus and Brassicas as well as Arachis will be used to show the versatility of methods and concepts regarding genome interactions. Identification of diversity and the relationships of species through phenotypic studies and sequence or other DNA-based markers are required. Knowledge of genome evolution and diversification mechanisms is helpful to understand the basis of variation. We will discuss repetitive DNA, representing the majority of the genome in most crop species, and how both retroelements and satellite DNA evolve and can be used as markers, including in orphan crops. Once identified, diversity can be exploited in new crops by synthesizing new hybrid species, by chromosome engineering or by transgenic strategies. Fluorescent *in situ* hybridization is valuable to characterise and track alien recombinant chromosomes in breeding lines for selection of improved traits. Genomic DNA and repetitive DNA probes can determine genome origin, identify chromosomes and chromosome regions and follow them through breeding pedigrees and crosses. The concept of superdomestication involves systematic identification of needs from crops, followed by finding appropriate characters and bringing them together in new varieties, and is certain to continue to deliver the new genotypes and farm products needed.

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Notes

Retrotransposons in *Arachis* – structure, genomic context, and phylogenetic classification

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The genetic order of low copy DNAs is well conserved between the A and B genomes in peanut. Main differences between the two genomes are based on substantially diverged repetitive DNAs, including transposable elements. We have isolated and characterized retrotransposon sequences from both genomes and present an in-depth analysis of two *Arachis* representatives of the Ty3-*gypsy* and Ty1-*copia* superfamilies, namely the elements FIDEL and *Matita*. Both elements not only differ in their structure, they also show different integration patterns regarding chromosomal and genomic distribution. FIDEL is a high copy *gypsy* element and is most closely related with the *Athila/Calypso*-group of retrovirus-like retrotransposons. It is more frequent in the A- than the B-genome and exhibits dispersed distribution in euchromatin, and absence from centromeres, telomeric regions, and the nucleolar organizer region (NOR), as reveald by FISH. Phylogenetic analysis of reverse transcriptase sequences showed its distinct evolution in the putative ancestor species. In contrast, *Matita* belongs to the *copia* superfamily, has moderate copy numbers, and is characteristically located on the distal regions of chromosome arms of both genomes. Phylogenetic analysis and molecular dating suggest that the distribution of the elements stems from the date of their last major bursts of transposition activity, which was estimated at around (*Matita*) and after (FIDEL) the time as the evolutionary divergence of the A- and B-donor species some 3-4 million years ago. For both elements a significant tendency of being more likely integrated near the fast evolving disease resistance genes homologs than near evolutionary conserved genes was demonstrated.

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Financial support: EU INCO-DEV (ARAMAP: ICA4-2001-10072), the Generation Challenge Program (Project G3005.05, and TLI) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Current progress in drought tolerance work in peanut – Field and lysimetric assessments of germplasm

Notes

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Intermittent drought is the most important yield limiting factor affecting groundnut (*Arachis hypogaea* L) production in rainfed regions of Sub-Saharan Africa and south Asia. Two hundred and forty seven (247) lines from the reference set of groundnut were assessed under well-watered (WW) and intermittent water stress (WS) in India and Niger for two years following same protocols. Yield differences within locations and years were mostly explained by genotypic effects, although genotype-by-treatment interactions were also significant, with pod yield under WW and WS being significantly related in both years (India, $r^2 = 0.42$ and $r^2 = 0.50$; Niger, $r^2 = 0.22$ and $r^2 = 0.23$). Within treatment across locations and years, pod and haulm yields were also explained by genotypic effects, and entries with consistent contrasting pod yield across locations under WS conditions were identified. By contrast, within location and treatment, pod and haulm yields were affected predominantly by genotype-by-year (GxY) effects, especially under WS. GGE biplot revealed two mega environments distinguishing India from Niger and also years in Niger, confirming the close interactions between the environmental conditions and the genotypic response to intermittent drought. Germplasm testing in a lysimetric system over two years showed large variation in the components of the Passioura equation, transpiration (T), transpiration efficiency (TE) and harvest index (HI). Although yield differences were mostly explained by HI, yield differences unexplained by the HI index were closely related to T and TE, depending on water regime. Also, pod yield was negatively related to differences in leaf area under severe stress, but not under mild stress or WW conditions. Therefore, groundnut tolerance to intermittent stress relates to particular patterns of water used, related to leaf canopy development under stress, which may highly interact with the environmental conditions and explain the large GxY interactions.

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Notes

Identification of QTLs for Oil Content and Fatty Acid Composition in Cultivated Peanut (*Arachis hypogaea* L.)

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Peanut is one of the important oilseed crops in the world. The quality of the seed is immensely dependent on the fatty acid composition. Quantitative trait locus (QTL) mapping of oil content and fatty acids is the prerequisite for peanut molecular breeding. A total of 215 F_9 recombinant inbred lines (RILs) derived from the cross ZHENG 8903 × YUHUA No. 4 were used to undertake QTL analysis. The field trial was carried out at the experimental farm of Henan Academy of Agricultural Sciences (Yuangyang county, China). Oil content and fatty acids were tested by the Agricultural Products Quality Supervision and Testing Center (Zhengzhou) of the Ministry of Agriculture. The oil content was determined by the Soxhlet extraction method with technical standard GB/T 14772-1993. Fatty acids composition including oleic acid, linoleic acid, arachidic acid, stearic acid and behenic acid were determined by capillary gas chromatography analysis with technical standard GB/T 17377-1998. Polymorphisms in parental genotypes were evaluated with 1556 simple sequence repeat (SSR) markers, 85 (5.46%) of which were revealed to be polymorphic. A total of 61 SSR loci were mapped into 18 linkage groups (LGs). WINQTLCart 2.5 and QTLNetwork 2.0 softwares were employed respectively to analyze the genotyping and phenotyping data, and nine QTLs accounting for 5.13%–24.14% phenotypic variation and 7 QTLs explaining 5.6%–25% phenotypic variation in oil content and fatty acids were identified accordingly. Moreover, four SSR markers, ARS95, GC-105, GC-92 and GW-263, closely linked to respective traits loci (with the genetic distance ≤3.0 cM) were identified, which may facilitate the marker-assisted selection for oil and fatty acids content.

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Capturing genetic diversity from peanut wild relatives: Advanced Backcross QTLs analysis and CSLL construction

Notes

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Cultivated peanut is an allotetraploid harbouring limited genetic diversity, likely resulting from the combined effects of its single origin and domestication. Peanut wild relatives represent an important source of novel alleles that could be used to broaden the genetic basis of the cultigen. Using a synthetic amphidiploid we have applied an original breeding scheme to capture the genetic diversity remaining in peanut wild relatives. We first produced an advanced backcross population. We then conducted, under two water regimes, a detailed QTL study for several traits involved in peanut productivity and adaptation and analysed the QTLs distribution on subgenomes. We mapped a total of 95 QTLs in the two water treatments. About half of the QTL positive effects were associated with alleles of the wild parent and several QTLs involved in yield components were specific to the water-limited treatment. QTLs detected for the same trait mapped to non-homeologous genomic regions, suggesting differential control of trait in subgenomes as a consequence of polyploidization. A set of 122 Introgression Lines (IL) that offers an extensive coverage of the cultivated peanut genome with generally a unique fragment per line and overlapping fragments between contiguous lines was developed. The utilization of the IL population for QTLs validation, breeding and gene discovery is discussed. Our findings open new avenues for peanut improvement using wild relatives.

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Notes

Isolation and characterization of important genes toward improvement peanut resistance to *Aspergillus flavus*

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The primary objective is to enhance resistance of peanut to *A. flavus*. To search important genes related to resistance, three Mixed cDNA pools, one derived from eight organs or tissues of different growing stages such as root, stem, leaf, etc, and seedling, two derived from root or leaf with/without a variety of treatments including biotic/abiotic stresses, and from carp in developing pod attacked by *A. flavus* under drought condition, were prepared and subjected to 454 sequencing, producing 2,022,025 reads after quality trimming. 94,421 unigenes after assembling, running against uniprot on swissprot and 25 model or important plants unigene database on NCBI by tblastX, and a total of 101344 unigenes were come out when we assembled these 94,421 unigenes with 11,909 peanut unigene on NCBI and 37,916 TSA peanut mRNA contigs on the same webpage. A high density of microchip with 101,344 unigene probes were developed and were used to screen genes related to *A. flavus* by comparative hybridization using RNA samples from pods treated with drought, drought plus *A. flavus* strain and normal condition in late growth stage of peanut. 9973 gene with 3 folds up/down regulated expression were isolated induced by *A. flavus* inoculation. Among them, 47 NBS-LRR-like genes were screened out with 18 genes being up/down-regulated expression for more than 5 folds which were also characterized for their expression patterns. Inbrief, So many NBS-LRR genes being involved in *A. flavus* infection imply that resistance of peanut to *A. flavus* is polygene inheritance.

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Some biogeographic, genomic and speciation considerations on section *Arachis*

Notes

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Section *Arachis* has two tetraploid, 29 diploid and 8-10 new species. A new genome organization has been recently proposed for the section distinguishing five different genomes: A, B, D, F and K. Moreover, the A-genome species were segregated into three different karyotype groups. This organization is supported by cross compatibility, some molecular markers and by 5S rDNA sequences. Satellite DNA and transposable elements also support this genome organization. Biogeographic analysis demonstrates that most of the species have restricted areas, some of them with vicariant populations, but very few with relatively extended areas. Field collections showed that populations of each species live in very similar habitats and that species of same genome tend to be restricted to particular biogeographic regions: the K-genome in the Chaco, the F in the savannas of the Beni and the B and D in the San Ignacio Planalto. Within the A-genome, each karyotype group was associated to different areas: Pantanal, Chiquitania and La Plata River basin. Whenever two *Arachis* species were found in simpatry, they had different genomes. This geographical structure of the genomic and karyotype groups may be a consequence of the particular biology of *Arachis* species. Geocarpy and a high degree of autogamy could be factors that contributed to the maintenance of new alleles within populations. New populations established from one or very few fruits dispersed from original populations by hydrochory, would have been exposed to genetic drift and rapid fixation of alleles, which ultimately, may have conducted to rapid speciation in close allopatry.

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Notes

Dispersed and other repetitive DNA sequences in the peanut genome shown by BAC-FISH

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Knowledge of the chromosome organization in *Arachis* species is relatively limited and currently includes the localization of centromeres and rDNA sequences besides the mapping of a single LTR retrotransposon, FIDEL. The physical localization of genetically mapped sequences on the chromosome map is a strategic tool for tracking and exploiting recombination and genome evolution. The present work aimed to extend *Arachis* physical map in order to explore genome and chromosome evolution using i*n situ* hybridization technique to metaphases of *A. hypogaea* 'Tatu' with A-genome (*A. duranensis*) BAC clones containing single- or low-copy gene-coding sequences as well as abundant dispersed and tandemly repeated sequence motifs. Each BAC showed a characteristic hybridization distribution pattern, labelling mostly the A-genome origin chromosomes with AT-rich centromeric domains predominantly excluding the centromere region. In spite of the use of high concentrations of blocking, most signals were located at multiple dispersed sites. These observations indicate the complexity of the peanut genome and are being used to characterize the nature and evolution of highly dispersed repetitive DNA sequences, results that will give an insight into the large-scale organization of repetitive and genome-specific sequences. Further characterization of the BAC clones is in progress and will complement and assist next-generation genome sequencing approaches, where repetitive DNA is particularly challenging.

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Molecular breeding for foliar disease resistance and quality-related traits in cultivated groundnut

Notes

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Breeding for foliar disease resistance and quality in groundnut is important while considering the yield loss and confectionary use worldwide. Rust and leaf spots (early and late) are the major foliar diseases. High protein, high oil content and high oleic/linoleic (O/L) ratio are the important qualities for confectionary purpose. However, combining higher levels of resistance into high yielding cultivars with acceptable market traits continues to be very difficult. Interference among the diseases, multiple oligogenes controlling resistance and undesirable linkages of resistance with other traits makes the breeding cumbersome and difficult. Oil quality analysis is cumbersome, time consuming, high resource-demanding. Hence, genomics approach with molecular markers is vital in handling such traits. A total of 3,097 SSRs were screened on the parents, TAG 24 (susceptible to late leaf spot and rust, and low O/L) and GPBD 4 (resistant to late leaf spot and rust, and high O/L), of which the segregation data was obtained for 209 marker loci on 266 RILs of TAG 24 × GPBD 4. A total of 188 loci were mapped on to 20 linkage groups spanning 1922.4 cM. QTL analysis for late leaf spot resistance identified several major and minor QTLs (R^2: 3.27-67.98%). $QTL_{LLS}01$ flanked by GM1573 and pPGPseq8D09 had an R^2 of 10.27-62.34%, and was stable across seasons. This is the first QTL for LLS resistance reported for the cultivated groundnut. Inclusion of additional markers in this study not only reconfirmed the previously identified and validated major QTL for rust, but also reduced the marker interval distance. Analysis for the quality-related traits revealed a total of 34 QTLs of which six were major (R^2: 10.25-25.94%) and 28 were minor (R^2: 3.20-9.86%). *ahFAD2A* allele linked with O/L ratio revealed a R^2 of 10.67-25.94% for oleic acid, linoleic acid and O/L ratio. These QTLs/markers are being validated and characterized with different genetic backgrounds for use in molecular breeding.

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Notes

Genetic engineering of groundnut for crop improvement: Current status and future prospects

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Peanut or groundnut (*Arachis hypogaea* L.) is a key commodity in the livelihoods of the rural poor in the semi-arid tropics. Several biotic and abiotic constraints, lack of high yielding adapted cultivars and aflatoxins adversely affect its productivity, quality and international trade. Inadequate resistance in the available germplasm for some of these constraints necessitates the use of modern biotechnological approaches including transgenic technology which provides an attractive alternative for the development of enhanced germplasm. Extensive efforts have been made at ICRISAT to develop efficient tissue culture and *Agrobacterium tumefaciens*-mediated genetic transformation protocol for peanut by using the cotyledon explants from mature seeds. This protocol is highly efficient and available for routine applications including the development of marker-free transformants for the recovery of clean transgenic events at high transformation frequencies (50-70%). A pipeline of genetically engineered peanuts for several traits are in different stages of development and validation. These include resistance to viruses like the *Tobacco streak virus* and the *peanut bud necrosis virus*, resistance to *Aspergillus flavus* and aflatoxin contamination, tolerance to drought stresses, and nutritional enhancement by the over-production of ß-carotene. A major emphasis is on robust phenotyping of these events under greenhouse and contained field conditions, and translational research for product development and commercialization. These strategies will allow effective use of the transgenic technology in conjunction with conventional plant breeding for sustainable crop improvement in the dryland tropics of the world.

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Global transcriptome analysis of peanut wild species under biotic and abiotic stress

Notes

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Legumes are an important source of protein for humans and livestock. Cultivated peanut (*Arachis hypogaea*) is one of the most widely grown grain legumes in the world, thanks to its high protein and unsaturated oil contents. Worldwide, the major constraints to peanut production are drought, foliar fungi and nematodes. Wild *Arachis* species, which are exclusively South American in origin, have high genetic diversity and have been selected during evolution in a range of environments and biotic stresses, constituting a rich source of allele diversity. *Arachis stenosperma* harbors resistances to a number of pests, including *Meloidogyne* spp. and foliar fungi, whilst *Arachis duranensis* has shown high tolerance to hydric stress. A 454 "GSFLX" transcriptome analysis from cDNA collections from multiple tissues and individuals generated a total of 7,814 unique sequences for *A. stenosperma* and 12,840 for *A. duranensis*, of which 29% had no match in the public database. This data was used to sample large numbers of expressed genes and also to detect simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs), as these species are parentals for reference mapping populations. Additionally, a deep coverage of transcripts produced during the pathogenic interaction of *M. arenaria* and *A. stenosperma* was conducted using Illumina Hi-Seq 2000, resulting in 115 million reads of which a significant number were found to be differentially expressed in each of the four time points of the interaction analyzed. This study should facilitate basic and applied research on the genetics and evolutionary studies of peanut, contribute to the development of molecular markers for other legumes and facilitate comparative genomics and the study of adaptive variation across the genus.

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Notes

Prevalence of aflatoxin contamination in groundnut value chains and strategies to enhance food safety in Mali

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Aflatoxin contamination is the most important food safety concern in several important crops and poses a threat to the health of consumers and the economic well-being of poor farmers in Mali. To better understand the prevalence of aflatoxins in groundnut value chains in different agro-ecological zones, investigations were carried out during 2009-10. Groundnuts taken at harvest from 90 farmers fields in 90 villages, in three agro-ecological zones, were tested for aflatoxin contamination. We observed the highest contamination, 172 μg/kg, in Kolokani (the most drought prone of the areas sampled) followed by 76 μg/kg and 35 μg/kg in Kita and Kayes, respectively. When the harvested produce in the farmers' granaries was, then, monitored monthly, analysis of samples revealed that aflatoxin content increased gradually from December to April, in all three locations. During 5 months of storage in farmers' granaries, the recorded increase in toxin content ranged from 261 to 521% over all three regions. Using the databases generated, maps were produced for Kolokani, Kita and Kayes Regions showing high aflatoxin prone areas at harvest and in storage, in all 90 villages. Analysis of nearly 4500 groundnut samples collected at regular intervals from traders, processors, wholesalers, and retail markets revealed no exception to the prevalence of unacceptably high levels of aflatoxins. Another phenomena was observed in groundnut "paste" which showed extremely high levels (>300 μg/kg) of aflatoxin in the majority of samples. Groundnut paste is prepared from poor quality groundnuts (after sorting kernels for the market), leading to high aflatoxin concentrations. We devised strategies and demonstrated to farmers integrated management technologies which address pre-harvest issues that will mitigate the toxin build up. The demonstration trials on integrated management of aflatoxins over the three locations showed 50-95% reduction in toxin contamination in farmer fields. Post-harvest management strategies were evaluated in the farmers' storage units, using double bagging of pod/seed and improved granaries, and led to aflatoxin reduction ranging from 68 to 99% reduction in aflatoxin content over the traditional methods. Overall, 4923 samples were analyzed in which aflatoxin contents ranged from 0 to 2231 μg/kg, with a mean of 146 μg/kg, for the country.

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Current Status and Objectives of Nursery El Carmen Peanut Breeding Program

Notes

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Nursery El Carmen is the leading Argentine breeder of new peanut cultivars, which for the last 15 years have been at the forefront of Argentina's peanut seed market. El Carmen released one the first Argentine runner cultivars and was the first domestic nursery to market High Oleic cultivars in 2003. El Carmen's seeds are also grown in Brazil, Nicaragua and other Latin American countries.Currently, around 75% of the peanuts grown in Argentina are "Granoleico", the first High Oleic peanut cultivar released by El Carmen. This fact represents a real weakness of the Argentine peanut industry for the last few years' slow genetic advances, and particularly the risk born by the genetic uniformity of the crop.The blueprint we designed to overcome this shortcoming is founded on two sets of breeding objectives. On one hand, the short term objectives based upon the traditional breeding schemes: *i)* seed shape and size, *ii)* length of growing cycle, *iii) Sclerotinia* blight tolerance, *v)* field and processing plant yield, *vi)* fat composition, etc.On the other hand, our long term broad strategic objective is to enrich the crop genetic base by the introgression of wild germplasm. Currently, we are developing breeding and mapping populations based on *Arachis correntina*, *A. Cardenasii* and *A. batizocoi* amphidiploids crossed with *A. hypogaea*. As middle to long term objectives, we expect to get *i)* foliar and soil born fungal disease resistances, from *A. correntina* and *A. Cardenasii*, *ii)* higher yield potential, and *iii)* drought tolerance.

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Notes

Genetic relationships in the genus *Arachis* section *Arachis* based on molecular data

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The genus *Arachis* is native to South America and contains 80 described species, assembled into nine taxonomical sections. The section *Arachis* is of special interest because it includes *A. hypogaea* and its wild relatives. Due to the narrow genetic base of cultivated peanut, wild relatives are important sources of useful genes. The knowledge of their genetic relationships is important for their efficient use, and to understand the evolution of *Arachis* species. We have analyzed intra- and interspecific relationships among accessions of all but two species of section *Arachis* using microsatellites and single-copy gene sequences. Both analyses showed clear groupings of species according to their genomes, corroborating the cytogenetic data. The resulting grouping of accessions and species also supports previous taxonomic classifications. Interestingly the D-, F-, and K-genome species were more closely related to the A than to the B genome species. Phylogenetic analysis with gene sequences enabled the comparison of the wild diploid species with the two genome components of the tetraploids in separate. The A genome component of *A. hypogaea* and *A. monticola* was placed in a clade that also contained the four accessions of *A. duranensis* and no other A genome wild species. The B genome component of the tetraploids formed a monophyletic clade, closely related to the only known accession of *A. ipaënsis*. These results strongly support the now well accepted hypothesis that *A. duranensis* and *A. ipaënsis* were the genome donors to *A. hypogaea*. They also corroborate the assumption that the tetraploid species originated from a single event of allopolyploidization or, if from multiple events, always involving the same parental diploid species.

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Development and Use of Molecular Markers to Accelerate Peanut Cultivar Development

Notes

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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 at Tifton 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. With the goal to pyramid nematode resistance and the trait for high oleic to linoleic acid ratio in seeds (high O/L), nematode resistant cultivar Tifguard was used as the recurrent female parent and high O/L cultivars Georgia-02C and Florida-07 were used as donor parents for the high O/L trait. >Tifguard High O/L= was generated through three rounds of accelerated backcrossing using BC_nF_1 progenies selected with molecular markers for these two traits as the pollen donors. Selfed BC_3F_2 plants yielded marker-homozygous individuals idnetifed as Tifguard High O/L, compressing the cultivar development process to less than 3 years. The accuracy of marker-assisted selection (MAS) was confirmed by phenotyping a subset of $F_{2:3}$ populations from both parental combinations. Once additional molecular markers linked with traits of interest are designed to be compatible with high-throughput screening platforms, MAS will be more widely integrated into peanut breeding programs.

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Notes

Broadening the genetic base of peanut: Introgression of wild *Arachis* resistance genes using the Tetraploid Route with the aid of molecular and cytogenetic markers

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Peanut diseases are the most important reducers of crop yield. Wild species harbor resistances against pests and diseases, but are agronomically unadapted, therefore little used in breeding. In addition, they are almost all diploid, while *A. hypogaea* is an allotetraploid. Here, we report the introgression of wild resistance genes into cultivated peanut by the creation of synthetic tetraploid donors. The initial interaction between the diploid *A. stenosperma* and late leaf spot, rust and nematodes were characterized by electron and light microscopy. Fungi were suppressed at early stages of germination, and nematode infection was also prevented at pre-penetration step. Gene expression analyses identified genes responsive to these biotic challenges. Late leaf spot resistance QTLs were identified close to RGAs on an *A. duranensis* x *A. stenosperma* cross. *Arachis stenosperma* was identified as a very promising source of disease resistance for peanut. In order to enable the introgression of wild genes into breeding populations, synthetics were obtained from *A. batizocoi* x *A. stenosperma, A. gregoryi* x *A. stenosperma, A. batizocoi* x *A. duranensis* and *A. ipaënsis* x *A. villosa* crosses. Synthetics derived from *A. batizocoi* as B-genome donor and their hybrids with cultivated had surprisingly high viability and fertility rates. Synthetics also presented high levels of resistance to rust. Hybridizations of the 74 synthetics produced here with cultivated varieties are underway. The introgression of genome regions is being monitored cytogenetically using DAPI-banding and genomic *in situ* hybridization (GISH) and by using molecular markers.

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Genetic and genomic resources of peanut to enable the use of wild relatives for crop improvement

Notes

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Peanut is an allotetraploid (AABB genome) with a narrow genetic base. Difficulties in the development of polymorphic DNA markers, and the complexities of tetraploid genetics hindered the development of genetic and genomic tools for peanut for some time. To help overcome these difficulties we developed diploid mapping populations and BAC libraries from the wild ancestors of cultivated peanut, and a tetraploid mapping population that incorporated high polymorphism from wild species. These populations together with new molecular markers allowed the generation of high-density genetic maps that are integrated with the genetic maps of other legumes. They show high synteny between the A and B peanut genomes that diverged from each other four million years ago; and high synteny with other legumes, from which peanut diverged 55 million years ago. Repetitive DNA of the peanut genomes, however, diverged strongly between the A and B and different wild species genomes. Some evidence suggests that this fast evolving component of the genome is linked to the evolution of new disease resistances that are markedly diverse in wild species. Whilst wild peanut species harbor resistances against pests and diseases, they are agronomically unadapted and almost all diploid, therefore their use in breeding is challenging. We investigated the potential of the use of wild-derived synthetics as donors of disease resistance to cultivated peanut. The synthetics showed a degree of resistance to late leaf spot and rust. Using genetic mapping we identified QTLs for late leaf spot, and by genotypic and phenotypic selection, we developed agronomically adapted peanut lines with enhanced resistances.

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Notes

Molecular and physiological approaches to improve abiotic stress tolerance in groundnut (*Arachis* sp.)

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To gain insights into molecular mechanisms of tolerance to water-deficit and heat stress, we screened the U.S. mini-core peanut germplasm collection and identified accessions with contrasting stress response phenotypes related to physiology, harvest index, and yield. Subsequently, we measured changes in the transcriptome and proteome of leaf, root, and seed under deficit irrigation and in response to heat stress acclimation. We identified a number of stress-responsive genes and proteins showing genotype-, tissue-, and time-specific response patterns. In addition to transiently stress responsive genes and proteins, profiling studies revealed a subset of genes that were 1) constitutively different between the tolerant and susceptible plants, i.e. different under control conditions and 2) differentially expressed following a return to optimal conditions and associated physiological recovery. For latter group, we noted that a number of genes that remained differentially expressed in the susceptible genotype were ones that were initially identified as constitutively different between the tolerant and susceptible genotypes under control conditions. We hypothesized that exposure of the susceptible genotype to a pre-acclimation stress (either water-deficit or high temperature) period followed by a return to optimal conditions would result in those plants responding in a manner similar to that observed for the tolerant genotype. That was indeed the case both physiologically and to an extent, transcriptionally. There were marked differences between acclimated and non-acclimated plants, and again "constitutive" differences between the genotypes. We will report the findings of this study, along with results from field trials of selected germplasm and current metabolome profiling data.

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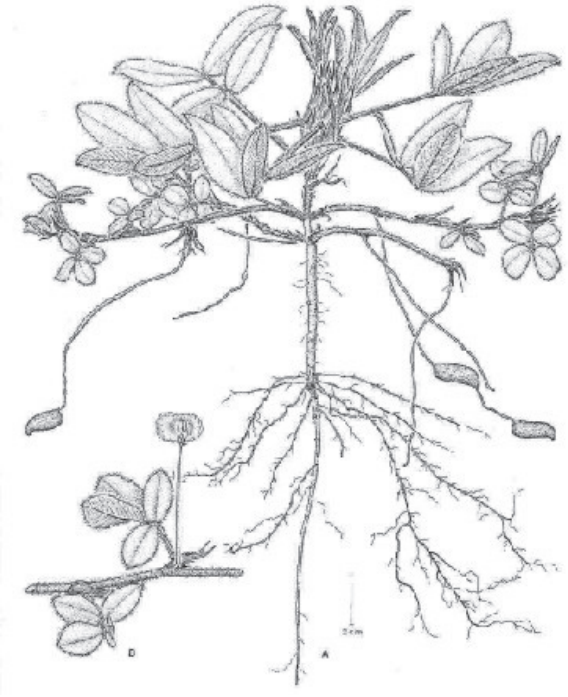
Abstracts

Posters



Peanut genes involved in oleic acid biosynthesis expression profiling in developing seeds at different reproduction stages

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The oleic acid content are important features in determining peanut (*Arachis hypogaea*). Oleic acid biosynthesis requires the coordinated activity of a large number of enzymes for the formation of unsaturated fatty acids and their further transformation in several aliphatic compounds.The objective of this study is to isolate genes related to oleic acid metabolic pathway in *Arachis hypogaea*. Compared with the Arabidopsis Lipid Gene (ALG) database (http://www.plantbiology.msu.edu/lipids/genesurvey/index.htm) 216 candidate genes that may play a role in fatty acid synthesis, regulation and transport were isolated from Suoleic expressed sequenced tag (EST) cDNA libraries. To identify whether the interesting candidates were also play an important role in oleic acid synthesis, regulation and transport , we measured the level of expression of 216 genes between the Suoleic(Hight oleic content cultivar) Zhenzhuhei (Moderately oleic content cultivar) Yueyou7(Low oleic content cultivar) in five different reproduction stages by performing microarray experiments. A total of 28 up-regulated genes (log(2) ratio > 1) were detected in Suoleicg Compared to Zhenzhuhei and Yueyou7. The top eight up-regulated genes were selected for validation of their expression levels by using RT-PCR and QRT-PCR. It implied that these genes may be related to the oleic acid synthesis. Although the selected genes identified by microarray analysis were validated by real-time PCR but further investioations are needed to characterize each of these genes. In this research, a proteomic approach was also used to investigate the changes of the five different reproduction stages by two-dimensional electrophoresis (2D) gels.

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Notes

Notes

The races of peanuts of Peru

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In Peru, there are **47** races pertaining to 5 botanical varieties:
13 Corresponding to subsp. *hypogaea* var. *hypogaea*
2 to subsp. *hypogaea* var. *hirsuta*
15 to subsp. *fastigiata* var. *fastigiata*
15 to subsp. *fastigiata* var. *peruviana*
2 to subsp. *fastigiata* var. *aequatoriana*

The collection of the Peruvian peanuts in the Farming Experimental Station of Manfredi, began in 1955 with the introductions of A. Krapovickas, increased in 1957 with the material sent by B. Mazzani, arriving to be increased remarkably with the samples obtained during the prospections realised in 1981 by E.C. Simpson, J.R. Pietrarelli & H. Or. Zurita.

The identification was realised on the base of the analysis of the type of plant, carries and ramification, characters of the fruit and color of grains

The type most common shortage in archaeological rest was the one that belongs to the *hypogaea* subspecies var. *hirsuta* Köhler whose fruits are very different from all the well-known ones.

In the Andean countries there is a regionalisation of the local races declares. Ecuador with **50** and Peru with **47** share two races. Between Peru and Bolivia with **61**, we know only three races common.

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New species of *Arachis* from the Bolivian Chiquitanía

Notes

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Chiquitanía is the eastern region of Bolivia in which a large ecozone is formed by the confluence of three different hydrographic basins and three very diverse phytogeographic regions. Field exploration until 2000 were mainly done using local flights to join distant points because of the many difficulties found for terrestrial displacement during the rainy season. Between the years 2002 and 2010 new expeditions were done thanks to a collaboration established between Bolivians and Argentineans to explore the area. The first objective of this collaboration was to intensify the collection of wild *Arachis* species in the search of materials that will help to reconstruct the evolutionary history of the Section in the region and to increase the allelic diversity available in the A and B genomes for potential introgressions to the cultivated peanut, *A. hypogaea* (AABB). So far, seven new species have been recognized and two new records were found for Bolivia. Among the new species, three belong to the A genome (perennials), two to the B genome (annuals) and one probably of K genome (annual). The new records for Bolivia are *A. hoenei* y and *A. glabrata*. The latter evidences the presence of section Rhizomatosae also in Bolivia. The botanical collections carried out in Bolivia in the last decade have identified the east of this country as one of the richest regions in *Arachis* species and demonstrated the immediate need to intensify field explorations in the search of new wild *Arachis*.

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Notes

Conventional breeding approaches to speed up release of new peanut varieties

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Reducing the time taken from first cross to commercial release of new peanut varieties is a key objective of global peanut breeding programs. While modern gene technologies, such as molecular markers, often purport that breeding can be made more efficient and faster, it is still possible to significantly speed up the breeding process using novel but complementary conventional breeding approaches. In the Australian peanut breeding program we have been able to significantly reduce the time to develop new superior varieties. A recent release (Tingoora) has only taken 6-7 years from initial cross to commercial release, in stark contrast to the mid 1990's when it took the program more than 13 years to release a new variety. The key factors associated with the reduced time to release included (a) faster generation time via the use of winter nurseries in North Queensland which nearly halved the inbreeding, selection and regional testing phases of the program. Winter seed production also allowed more rapid seed multiplication and saved nearly 3 years in the variety development program; (b) closer collaboration between the breeding program and variety commercialiser (e.g. PCA) meant it was able to take on greater commercial risk to enable the fast tracking of commercial scale seed increase. This meant that significant time was saved in increasing the new variety to commercial scale seed quantities; (c) new speed breeding techniques using a 24h continuous light system can result in significantly reduced generation time (140 to 85 days). A single seed decent breeding strategy can then be used to fast track the inbreeding process from F2 to F5 in a single year. This strategy can save a further 1-2 years and potentially allow an 'initial cross to commercial release' time of only 5-6 years.

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Financial support: Grains Research and Development Corporation, Peanut Company of Australia, AgriSciences Queensland

Use of wild Arachis species for peanut (*A. hypogaea* L.) improvement at North Carolina State University

Notes

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At NCSU, we maintain a large collection of wild *Arachis* species with a goal to preserve *Arachis* genetic resources and to utilize them for peanut improvement. Wild *Arachis* species have been documented as sources of resistance to common peanut pathogens and insect pests. In North Carolina, Early Leaf Spot (ELS) and Tomato Spotted Wilt Virus (TSWV) have been the most persistent disease problems although Late Leaf Spot (LLS), Cylindrocladium Black Rot (CBR), Sclerotinia Blight (SB) have also been damaging. We have evaluated wild *Arachis* species for disease and pest resistances using convention laboratory/greenhouse inoculations and very high levels of resistance/immunity has been identified for many pathogens and insect pests, leading to the development of several germplasm and advanced breeding lines from interspecific hybridization. Among the advanced interspecific hybrid-derived breeding lines, SPT 06-06 and SPT 06-07, are highly resistant to ELS and SPT 06-07 is also resistant to TSWV and SB. Attempts to link molecular markers with TSWV and SB resistance are currently underway. Although no single line had resistance to all of the above diseases, it is encouraging that some of the breeding lines, particularly, SPT 06-07, exhibited resistance to three of the four diseases. These results suggest that some of these breeding lines maybe useful as parents in peanut breeding programs.

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Notes

Deno nove characterization of peanut transcriptome during gynophore development

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As one of the most important oil crops, peanut (*Arachis hypogaea* L) is wildly grown in China, India and the United States. It is different from most of other plants, peanut flowers above the ground but fruits below the ground. It is interesting to study the mechanisms of gynophore development. Cloning and functional study of specific genes involved in gynophore development maybe provide important information during this process. The genomics research of peanut is far behind other plants such as soybean. There are only 87688 ESTs of cultivated peanut were deposited in the database and even less ESTs from peanut gynophores. In this study, we collected gynophores at different growth stages, which include gynophores before and after soil penetration. RNA was extracted from these plant materials and transcriptome was analyzed by Illumina HiSeq™ 2000. Totally we acquired 13,293,536 raw reads with average length 90 bp. These reads were assembled into 72527 unigenes (mean size: 394 bp) using SOAPdenovo software. The unigenes were annotated by successively BlastX against Nr, Swiss-Prot, KEGG and COG Database. The results showed that these genes involved in different biological function, such as metabolic, plant-pathogen interaction, biosynthesis of phenylpropanoids and flavonoid, cricadian rhythm and fatty acid metabolism. And interestingly, many genes related the biosynthesis of plant hormones were identified. This study provided valuable information for further study on peanut gynophore and pod development.

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Financial support: National Science Foundation of China (30871324, 31000720)

Advance of Peanut Omics and Biotechnology in China

Notes

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Peanut is one of the most important oil crops in the world, both for vegetative oil and as a protein source. Improvement of peanut oil content and quality, protein content and quality is important for both breeders and consumers. Biotechnological approaches such as marker assisted selection (MAS) and gene engineering provide promising ways to increase crop productivity, either through improved seed yield and quality or stress resistance. Biotechnological based improvement of peanut requires the understanding of the mechanisms that control the agronomical traits. Therefore, it is crucial to understand the molecular mechanisms of seed storage protein production and oil accumulation through functional genomics and proteomics. The identification of genes that control important agronomical traits, the understanding of gene regulation and regulation of metabolic pathways, ways of delivering genes or small RNAs into peanut plants as well as the establishment of MAS system are equally important. Because of these requirements, extensive studies in China have focused on peanut functional genomics and biotechnology, and have made great strides during the past decades. The following research areas are emphasized in this review: EST sequencing; peanut transcriptome study; gene cloning; gene expression profiling and gene regulation; molecular marker development and application; peanut in vitro regeneration and gene transformation; peanut miRNAs as well as peanut proteomics studies.

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Financial support: National Science Foundation of China (30871324, 31000720)

Notes

Identification of peanut (*Arachis hypogaea* L.) miRNA targets through degradome sequencing

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Cultivated peanut (*Arachis hypogaea* L) is one of the most important oil crops. It is grown in more than 80 countries around the world. MicroRNAs (miRNA) are a new class of small, endogenous RNAs that play a regulatory role by negatively affecting its target gene at the post-transcriptional level. It has been shown to control numerous genes involved in various biological and metabolic processes. In the previous study, we cloned and identified 75 conserved miRNAs and 14 novel miRNAs families from cultivated peanut by high-throughput sequencing. By preliminary bioinformatics analysis, some miRNA target genes were predicted, while most of the miRNA targets were unknown. Here, we employed a new experiment method, degradome sequencing, to directly detect miRNA cleaved sequences and identify miRNA targets. A degradome library was generated from mixed-tissue (roots, stems and leaves) of cultivated peanut, and sequenced by high-throughput sequencing techniques. We identified novel miRNA targets by degradome sequencing together with sequence information of peanut ESTs in NCBI dbESTs and peanut transcriptome results. These targets were annotated by BlastX online. The results showed that the identified miRNA targets were mainly associated with plant growth and development, as well as stress resistance, for example, growth-regulating factor, L-galactono-1,4-lactone dehydrogenase, stress- related protein, ubiquitin fusion protein, aquaporin protein and thioredoxin were found in the list of target genes.

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Financial support: National Science Foundation of China (30871324, 31000720)

Cloning and expression analysis of flowering regulating genes in peanut

Notes

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Most of the peanut flowers formed during the late stage can not develop into gynophore or mature seeds. Reduce the flower formation at this stage may channel more energy and photosynthesis products to the developing seeds. Biotechnology together with the understanding of flowering mechanism in peanut may provide a way to control peanut flower development. So, it is of great importance to study the genes that function as flowering promoters or repressors. The FT/TFL1 gene family encodes proteins with similarity to phosphatidylethanolamine binding proteins, which in Arabidopsis includes FT and five other related genes, TSF, TFL1, BFT, ATC and MFT. FT, TSF and MFT seem to function as integrator of flowering time pathways and appears to promote flowering redundantly. While TFL1 controls the length of the vegetative phase and delays the flowering transition. In this study, by cDNA library screening and homology based cloning, the candidate genes of *Ah*FT, *Ah*FTL (*Ah*FT Like), *Ah*TFL1 and *Ah*MFT were isolated. Amino acid sequence comparisons revealed that the critical amino acid residues (Tyr85/Gln140 in *Ah*FT versus His91/Asp147 in *Ah*TFL1) were highly conserved. The genomic structure of *Ah*FT, *Ah*MFT and *Ah*TFL1 showed a conserved exons and introns patterns to compare with genes from other species. FTL protein was identified from leguminous plants including peanut, soybean and medicago, but not in other plants. The expression patterns of these genes were analyzed. This study provided important information of peanut flower development and flowering control through biotechnological approaches.

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Notes

Tagging of rust and late leaf spot disease resistance gene and identification of functional diversity with genic molecular markers in cultivated groundnut

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Development of molecular markers for different economic important traits in cultivated groundnut has progressed at slower pace. Although many genomic SSR markers in turn genetic linkage maps were developed in cultivated species, such of the linkage maps need to be saturated. Availability of large number of ESTs in Genbank opened up the possibility of map saturation and identification of closely linked markers for agronomic traits. EST-SSR markers are also considered as genic molecular markers (GMMs). In this study, more number of GMMs were developed and used for functional diversity in selected groundnut genotypes and genetic mapping of rust and late leaf spot disease (LLS) resistance using a RIL population derived from VG 9514 X TAG 24 cross. The 26 GMMs detected diversity in activities of transcription factor, ribonuclease 2, oleyl-phosphatidyl choline desaturase, translation initiation factor (eIF-1A) as well as differences in a putative senescence associated protein, a hypoxia induced protein and in two resistance gene analogues. Further, through genetic mapping technique a unique EST-SSR marker, GO340445 was found closely linked to a rust resistance gene at 1.9 cM distance. Similarly, three functional GMMs, Cer 2, RGC 2 and GO340073 were associated with late leaf spot resistance in groundnut. The strong genetic linkage of GO340445 with rust resistance gene and functional association of Cer 2, RGC 2 and GO340073 with LLS resistance rendered these markers as a suitable candidate for marker assisted selection in groundnut. The physical closeness of GO340445 will help in future to screen the BAC clones that contain the rust resistance gene in groundnut and isolate rust resistance gene sequence.

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Developing transgenic resistance in cultivated peanut (*Arachis hypogaea* L.) to peanut-stem- and peanut-bud-necrosis viruses

Notes

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Stem- and bud-necrosis are the major virus diseases of peanut crop in India. When epidemic, these diseases cause to huge economic losses which may be as high as 80% if infection occurs early in the crop season. Currently, in India peanut varieties resistant to these viruses are not available. Sources of genetic resistance to these viruses are not available in the germplasm collection for their exploitation through conventional breeding approaches. To address this issue, an attempt was made to develop transgenic resistance in peanut plants using coat-protein/nucleocapsid genes of these viruses. Three gene-constructs containing, i) coat-protein (CP) gene from PSNV (TSV); ii) nucleocapsid (NC) gene from PBNV; and iii) both CP gene of PSNV and NC gene of PBNV (dual construct) were developed. Using the protocols reported earlier (Radhakrishnan *et al* 2002), transformation of peanut cultivars K 6 and K 134 was attempted. Confirmed events - 73 for CP gene of PSNV, 5 for NC gene of PBNV and 19 for dual construct were obtained. RT-PCR analyses of the events revealed the expression of the gene(s). The transgenic plants did not show any symptoms of the disease(s) upon artificially challenging with virus(es) concerned while the wild type plants expressed the symptoms. The presence as well as the load of virus particles in both transgenic and control plants were confirmed by DAC-ELISA. Large-scale and detailed screening of the transgenic events, are in progress under containment.

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Financial support: Department of Biotechnology, Gov. of India

Notes

Effect of peanut wild genotypes, cultivar and amphidiploids on the development of *Enneothrips flavens* moulton (*Thysanoptera: Thripidae*)

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Peanut is a dicotyledonous plant of the family Fabaceae, subfamily Faboideae, genus *Arachis*, which comprises about 80 species distributed in Cerrado biome and wide vegetation environments. Among the wild species of *Arachis* there are several promising genotypes for studies about resistance to insects. Thus, this work aimed to evaluate the effect of six peanut genotypes, four wild species, one amphidiploid (hybrids originated from two wild species of distinct genomes artificially tetraploidized by the use of colchicines) and one commercial cultivar, IAC Runner 886, on the biology of *E. flavens*, in laboratory. For the conduction of the experiment we used Petri dishes with softly moistened filter paper at the bottom where we put one young leaflet of each genotype in each dish. In the same plate we put seven adult thrips which were collected in the field, for oviposition, and they were removed after 24 hours. We used the experimental design of completely randomized blocks, with 30 replications. We evaluated the following biological parameters: duration of periods of egg incubation, first and second nymphal instars, pre-pupae, pupae, egg to adult, adult longevity and total viability. We observed in wild genotypes KG 30097 (*Arachis magna*), V 7635 and V 7639 (both *A. kuhlmannii*) a significant increase of the stages of the cycle of the insect, inferring that these genotypes have antibiosis-type resistance to this pest. In the other hand, the commercial cultivar IAC Runner 886 showed lower mortality rate and behaved as susceptible to thrips.

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Financial support: Capes,CNPq

Biological aspects of *Stegasta bosquella* (CHAMBERS, 1875) (Lepidoptera: Gelechiidae) on peanut wild genotypes, cultivar and amphidiploids

Notes

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In Brazil, several pests may attack peanut crop, and among these stands the peanut rednecked worm, *Stegasta bosquella* (Chambers, 1875) (Lepidoptera: Gelechiidae), which is considered one of the major pests of this crop. Thus, the aim of this work was to evaluate the effect of six peanut genotypes, four wild species, one amphidiploid (hybrids originated from two wild species of distinct genomes artificially tetraploidized by the use of colchicines) and one commercial cultivar, IAC Runner 886, on the biology of *S. bosquella*, in laboratory. For the conduction of the experiment we used Petri dishes with softly moistened filter paper at the bottom, where we put in each dish one young leaflet of each genotype and one recently hatched caterpillar from the stock rearing. We used the experimental design of completely randomized blocks, with 30 replications. We evaluated the following biological parameters: period and viability of larval, pupal and total stages, weight of caterpillars with 8 days-old, weight of pupae with 24 hours-old and adult longevity. We observed in wild genotypes V 7635 and V 7639 (both of wild species *Arachis kuhlmannii*) total mortality of the insects in pupae and adult stages, which showed antibiosis-type resistance to *S. boquella*. In total viability the amphidiploid V 6389 x V 9401 (*A. gregoryi* x *A. lineariforlia*) showed higher mortality rate, suggesting to be resistant to this pest. In the other hand, the commercial cultivar IAC Runner 886 showed lower larval, pupal and total mortality rates, therefore, susceptible to the peanut rednecked worm.

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Financial support: Capes,CNPq

Notes

Characterization of Brazilian accessions of wild *Arachis* species of section *Arachis* (Fabaceae) using heterochromatin detection and fluorescence *in situ* hybridization (FISH)

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Twelve Brazilian accessions of five *Arachis* species were characterized using FISH and heterochromatin detection. 5S and 45S rDNA loci, syntenic sites, and chromosomes with heterochromatic bands were observed. *Arachis glandulifera* V14730 presented eight 45S sites, two intense 5S sites and possibly two faint ones, seven chromosome pairs with mainly pericentromeric, but also interstitial heterochromatic bands, quite similar to the pattern described from Bolivian accessions. *Arachis valida* accessions V9153 and V13514 presented six 45S and two 5S sites, with synteny in one chromosome pair. Centromeric heterochromatic bands were absent, although a telomeric band could be present in one chromosome pair of V13514, as described from the type. Accessions of *A. gregoryi* V6389, V14743 and V14957 had two 5S and two 45S sites, not syntenic, similar to previous information on V14753. Five Brazilian accessions identified as *A. magna* did not show heterochromatic bands. All five presented two 5S sites, as for the type. They varied concerning 45S sites, two in V13748, V14727, V14750, four in V13765, six in V14724. None exactly repeated the 45S pattern of the type collection. Identified as *A. hoehnei*, accession V9146 presented four 45S and four 5S sites, in two syntenic chromosome pairs, and centromeric heterochromatic bands in all pairs. This accession shows a small chromosome pair, which however does not seem to behave as the A pair. It is possible that two types of plants, morphologically similar but with significant cytogenetic differences, are filed under *A. hoehnei*, of which those not corresponding to the type should be segregated.

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Financial support: EMBRAPA/CNPq

Towards ultra-dense genetic maps of peanut generated by sequencing diploid and tetraploid RIL populations and a peanut diversity panel

Notes

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We describe a project that will generate ultra-high density genetic maps through low coverage, shotgun sequencing of diploid and tetraploid mapping populations and of a peanut diversity panel. The diploid RIL mapping populations were generated at EMPRAPA and include the wild progenitor species of both, the peanut A and B genomes (AA pop: *A. duranensis*, *A. stenosperma*; BB pop: *A. ipaensis, A. magna*); the tetraploid mapping population is derived from a cross of a cultivated peanut (*A. hypogaea* cv. IAC Runner 886) and a synthetic amphidiploid of the two progenitor species. The diversity panel consists of 300 accessions selected based on SSR genotyping data to represent the diversity of the international peanut germplasm collection at ICRISAT. The gene space of the parental lines of each RIL population (to 20x coverage) and of each RIL and each accession of the diversity panel accessions (to 1x coverage) will be sequenced using an Illumina HiSeq. The data analysis will be carried out using similar approaches and genotype calling and mapping software developed for studies in rice. The SNPs identified in the diversity panel will be analyzed for linkage disequilibrium. The LD data will be used to refine the genetic bins generated from the RIL segregation data. The diversity panel analysis will also provide the foundation for GWAS studies and efficient QTL mapping. In addition, the project will both assist and complement the assembly of the reference genome sequence for peanut by providing chromosomal genetic coordinates for contigs and scaffolds.

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Notes

Variation in seedling morphology of wild *Arachis* species

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Available at the Embrapa Wild *Arachis* Genebank, germplasm of most *Arachis* species is periodically increased from cold stored seed. Since De Candolle's 1825 illustrated account, descriptions of seedling morphology only covered *A. hypogaea*. Disciplined observation of seedling morphology of accessions of all nine taxonomic sections revealed a broad array of alternative or additional characters, often peculiar to individual seedlings, or to selected accessions, but generally common to all accessions of a single species, or eventually shared by all analyzed species of a given section. Seedlings were obtained in a BOD germination chamber, on Germitest paper packages. The analysis was complemented by observation of voluntary seedlings in non-harvested pots of selected species. Laboratory screening accounts for most data, but greenhouse checking allowed for the distinction of most seedlings as phanerohypogeal, less frequently phanerogeal or phaneroepigeal. Variation was noticeable concerning presence and proportional length of a cotyledonary petiole, channeled x flat internal face of cotyledons, sometimes deeply sulcate, the relative length of epicotyl/hypocotyl, epicotyl hairiness, presence and distribution of anthocyanins, and opposite x alternate insertion of the first leaf pair. Association of channeled cotyledons with proportionately very long petiole in the first 3-4 leaves distinguishes members of *Procumbentes*, while most *Arachis* section species link a pubescent hypocotyl to opposite first pair of normal leaves. Adding novel features for characterization of species and accessions, seedling morphology emphasizes the heterogeneity of section *Heteranthae*, possibly a group of genetically divergent species, just showing morphological convergence for adaptation to the semi-arid environments of the Brazilian Northeast.

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Stigma tip morphology of *Arachis* (Fabaceae) species from seven taxonomic sections, with emphasis on sections *Extranervosae* and *Heteranthae*

Notes

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Wild *Arachis* species are exclusive to Argentina, Bolivia, Brazil, Paraguay and Uruguay. Brazil has 62 native species, all conserved and submitted to characterization at the Wild *Arachis* Genebank, in Brasília, representing the nine taxonomic sections. Morphology of the stigma tip varies in *Arachis*. It was analyzed in representatives of sections *Arachis* and *Rhizomatosae*, and differences have been associated to life cycles. But intensive variation was documented in *Caulorrhizae*, which only encompasses perennials. The characterization was extended, comparing morphology of the stigma tip of representatives of *Extranervosae* and *Heteranthae* to that of plants of five other sections, stressing differences and similarities, and correlating morphological characters and seed production to annual x perennial cycle. Stigmas were collected from plants cultivated at the genebank, clarified, conserved in 50% ethanol, stained and photographed. Length (μm) and density of trichomes were observed, and related to the specific life cycle. Records of 2005-2010 voluntary seed production in pots of the accessions tested were compiled, covering 34 species, comparing annual x perennial cycle (*t Student* test). Seed production did not vary as a function of life cycle (MANOVA F 1.201=0.06p=0.8; corrected for species and harvest year). The species analyzed of seven sections present obvious morphological differences concerning length and density of stigmatic trichomes. But variation of length x density could not be associated to life cycles of the species involved. However, stigma tip morphology is similar in representatives of sections *Extranervosae* and *Heteranthae*, and their pattern is reproduced by the genetically isolated *A. burkartii* of section *Rhizomatosae*.

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Notes

Identification and introgression of a major QTL for rust resistance in elite cultivated groundnut cultivars through marker-assisted selection

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Groundnut (*Arachis hypogaea* L.) is a major source of vegetable oil and protein. Rust, caused by *Puccinia arachidis,* is widespread in most of the tropical countries and severely affects the crop productivity and quality of the produce. Since, the trait is quantitative in nature and governed by recessive genes, mapping resistance genes/QTLs will be of high significance for groundnut breeding. In this context, using a recombinant inbred line (RIL) mapping population derived from the cross TAG 24 × GPBD 4, a total of 12 QTLs with 1.70-55.20% phenotypic variation explained (PVE) were identified using composite interval mapping (CIM). Interestingly, one major QTL ($QTL_{rust}01$), contributing upto 55.2% PVE was identified by both CIM and single marker analysis (SMA). The tightly linked SSR marker, IPHAHM103, with the $QTL_{rust}01$ was validated using a wide range of resistant/ susceptible breeding lines as well as another mapping population derived from the cross TG 26 × GPBD 4 segregating for rust resistance. Subsequently, marker-assisted introgression of this QTL has been initiated in three elite groundnut susceptible varieties namely ICGV 91114, JL 24 and TAG 24 using the donor GPBD 4 through marker-assisted backcrossing (MABC). IPAHM103 marker, together with four other markers identified in the same QTL region based on TG 26 × GPBD 4 populations are being used for foreground selection to identify heterozygous plants at F_1, BC_1F_1, BC_2F_1 and BC_3F_1 generations. While some flowers from the selected BC_2F_1 and BC_3F_1 plants were used for another round of backcrossing, some flowers were selfed to generate BC_2F_2 and BC_3F_2 seeds. Current status of MABC work will be presented in the meeting.

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Crop Tool Management / DSSAT genetic data on calibration of the Peanut for simulations of future scenarios

Notes

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The parameterization of the model DSSAT (Decision Support System for Agrotechnology Transfer) to the Brazilian reality is very important to obtain an instrument for monitoring agricultural crops and can predict future agricultural scenarios. Crop Management is a very important tool for gauging Genetic Data Peanut model by comparing the values observed in the field with the simulations for the following variables: critical short Day lenght below wich reproductive development progresses with no daylenght effect (hour), slope of the relative response of development to photoperiod with time (1/hour), time between plant emergence and flower appearence in photothermal days (ptd), time between first flower and first pod (ptd), time between first flower and first seed (ptd), time between first seed and physiological maturity (ptd), time between first flower and end of leaf expansion (ptd), maximum leaf photosynthesis rate at 30 °C, 350 vpm CO_2, and high light (mg CO_2/m^2 - s), specific leaf area of cultivar (cm^2/g), maximum size of full leaf (cm^2), maximum fraction of daily growth that is portioned to seed + shell, maximum weight per seed (g), seed filling duration for pod cohort (ptd), average seed per pod, time required for cultivar to reach final pod load (ptd). There are great possibilities for application of crop simulation models in technology transfer. The management of culture can be easily simulated, showing the result for producers in terms of production or profitability of the interactions that culture suffers from the environment over the cycle.

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Notes

Selection of *Arachis hypogaea* breeding lines for resistance to multiple foliar diseases

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Foliar diseases are major concerns for peanut production in southeastern Brazil. Developing resistant cultivars is of special interest, but its success faces two limitations: one is the quantitative inheritance of these traits and, the other, is that various diseases may occur at the same time along the peanut areas. Thus, breeding should consider possibilities of selecting for resistance to multiple pathogens. Early and late leafspot and rust were assessed in one hundred and ten advanced breeding lines in a field experiment without chemical control of foliar diseases, at Pindorama, SP, Brazil, using an augmented block design including seven control cultivars ranging from susceptible to moderately resistance, in five replications. Disease assessment was performed at three dates along the growing cycle and the data were used to estimate the area under the disease progress curve (AUDPC). Average AUDPC ratings for early and late leafspot and rust among the controls ranged from 79.6 to 265.5, 286.0 to 545.0 and 72.0 to 337.5, respectively. Considering simultaneous selection for the three diseases, ratings for the best ten lines ranged from 140.1 to 177.3, 72.0 to 105.8 and 72.0 to 132.8. The data indicate that the population studied contains lines with high resistance to late leafspot, bringing together moderate levels of resistance to early leafspot and rust.

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Genetic diversity of the gene Resveratrol Synthase in *Arachis* spp. from Embrapa active germoplasm bank

Notes

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Resveratrol is a fitoalexin from the stilbene family synthesized only by a few plant species such as peanut and grapevine. The production of Resveratrol is triggered by biotic and abiotic stresses and is directly linked to resistance to pathogens. Besides its role in plant defense, Resveratrol has a significant value in human health due to its anti-oxidantproperties.This fitoalexis is a product of the enzyme Resveratrol Synthase. In order to observe the diversity of the gene Resveratrol synthase in *Arachis*, eight different genotypes of *Arachis*were used in this study(twocultivated, five from wild species and one synthetic anfidiploid). Primers were designed for Stilbene Synthase genes of peanut from the Genbank. Amplicons were obtained for all genotypes. Sequence analyses revealed five distinct groups for this gene family. This diversity is randomly distributed within the genotypesstudied, suggesting that the allelic diversity is prior to the speciation events within *Arachis*. Further analyses will take place to study the expressionlevels of Resveratrol Synthase gene under stress conditions. This effort intends to highlight alleles leading to Resveratrol accumulation to be used in breeding programs, making use of diversity of wild *Arachis* relatives as a source of genetic variation.

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Notes

Development and characterization of highly polymorphic SSR markers for genetic analysis of *Arachis*

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Peanut (*A. hypogaea*) is a crop of economic and social importance, mainly in tropical areas, and developing countries. Its molecular breeding has been delayed by the shortage of enough polymorphic markers due to the low level of polymorphism, result of a narrow genetic basis. This is the result of *A. hypogaea*'s origin of one or few events of tetraploidization, and its isolation from the wild diploid relatives. SSR (microssatellite) markers have been shown to be very informative and transferrable between *Arachis* species. TC-repeat SSRs were found by several authors as the most polymorphic type. Additionally, long repeats were also found to be more polymorphic than short repeats. EST-based SSRs were generally also found to be less polymorphic than SSR markers developed from enriched genomic libraries. In spite of several efforts, the number of SSRs that are polymorphic for *A. hypogaea* is still small, creating the need for the discovery of more markers. In this work, a TC-repeat enriched genomic library was created, resulting in 303 TC repeats, for which 147 primers were designed. 94.6 % of the primers had positive amplification. These primers were evaluated in 24 genotypes encompassing all six botanical varieties of *A. hypogaea*, and included the wild tetraploid *A. monticola*, and two peanut landraces. The overall polymorphism was 59%, the highest rate found to date. These results are significant; the markers isolated here represent a significant addition to molecular markers in peanut.

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Characterization of duplicate genes involved in oil pathways of polyploid peanut

Notes

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Peanut is the fourth-largest oilseed crop in the world. Nevertheless, there has been relatively little molecular research on the biosynthesis and metabolism on fatty acids (FA) and oil related genes in peanuts. Also, studies sometimes ignore the fact that cultivated peanut is an allopolyploid organism with a whole duplicated genome (AABB). Here, we have identified and characterized the expression pattern of 11 duplicated genes associated with five protein families that represent key stages in the FA and oil biosynthetic pathway (FAD, SAD, DGAT, FATA and FATB). We measured the RNA expression levels of these genes in developing seeds of six genotypes, representing different marketing types, pod types, oil contents and FA profiles. We sampled seeds at four developmental stages (initial, expansion, breaker and full-ripe). Leaf and root tissues were used as controls. RNA expression was measured using quantitative RT-PCR. FA profiles for each Genotype × Stage were evaluated using GC-MS. Homeolog-specific analyses were performed either real-time PCR or MALDI-TOF mass-spectrometry assays performed using the Sequenom MassARRAY platform. For each family, we detected homologous genes that are seed-specific, non-seed-specific or not expressed in seeds. Significant differences between genotypes, time points and Genotype × Time Point interactions were found for all variables (mRNA level, homeolog-specific bias, FA profile). The greatest changes in genome-specific RNA expression levels were observed for seeds containing high levels of oleic-acid, seeds with high oil contents and seeds with low oil contents. This study provides an initial glimpse into pathways of oil biosynthesis during seed development in peanut.

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Notes

Identification of QTLs for drought tolerance across different locations and seasons using recombinant inbred lines in peanut (*Arachis hypogaea* L.)

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Peanut (*Arachis hypogaea* L.) is an important food and cash crop grown in semi-arid tropics (SAT) regions where intermittent drought spell affect adversely the yield potential of high yielding cultivars. Tolerance to drought is a complex trait and it has been very difficult to breed tolarant genotype through conventional breeding. However, recent advances in crop biotechnology have enabled identification and transfer of genomic regions associated with quantitative traits. Genotyping data was generated for 191 SSR marker loci and phenotyping was done at Bambey (Senegal) in 2009 and Sadore (Niger) in 2009 and 2010 for yield and yield components under fully irrigated and water stressed conditions on a recombinant inbred line (RIL) derived from the cross TAG 24 × ICGV 86031. QTL analysis using QTL cartographer and Genetotype Matrix Mapping (GMM) resulted in detection of 19 main effect (M-QTLs) and 18 epistatic QTLs (E-QTLs) for yield and yield component traits. Although trait heritability values were quite consistent but low for a given trait over locations and seasons, QTLs detected showed phenotypic variance explained (PVE) ranging from 4.33-11.38 %. Furthermore, detected QTLs were mostly environment-specific. Interestingly, co-localized QTLs for pod yield and shelling percentage were detected on the linkage groups 2 and 6. Also some QTLs for pod yield, haulm yield and SCMR detected are located on the same or adjacent regions of the linkage group 5. This study suggests deployment of modern approaches like marker-assisted recurrent selection to introgress small effect QTLs for breeding for drought tolerance in peanut.

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The Kayabi indigenous group and the peanuts – *Arachis hypogaea*. Some cultural and evolutionary aspects

Notes

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The Kayabi Indigenous group (Tupi speakers) has in the peanuts their main crop, with many landraces. The peanuts is involve in many parts of their culture, as myths, food, histories.Here we present three aspects of the use and management that they have with peanuts. First: the time of the cycle of each landraces differ. So, they use to plant each kind of landraces in different dates. First they plant the long cycle ones, successfully until the last with shorter cycle. This makes that all the landraces can be harvest all together, so they synchronize the harvest. The second aspect is about the use that they do with those plants that grows after the harvest, through the seed that "escape" in the field. That seeds, that had been broken in the plant'peg, they said that they taking care along their cycle and harvesting in the right moment, but will only use for food and never use it to create a new crop field, because, as they say, those seeds are "weak". Interesting, this management makes that occurs a directional evolution for genotypes with strong pegs, once the seed that they use to plant the new fields came from those plants that keep the fruits attachment in the plant, in the harvest moment, eliminating those genotypes with weak pegs. Third: They test different youth in the village, to see who will have "good hand" to plant peanuts. So they do a selection for who is aloud to plant peanuts and who is not.

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Notes

Potential of new *Arachis* amphidiploids as sources of resistance to leafspots and rust

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Development of amphidiploids have been proved to be an efficient strategy to allow introgression of various wild germplasm sources to *A. hypogaea* cultivars. The technique consists in crossing species of A genome with those of B genome, obtaining sterile F_1 hybrids, which are treated with colchicine, generating fertile amphidiploids. A number of them have been recently obtained and have been field tested for resistance to insects and foliar diseases at localities close to the commercial peanut regions in São Paulo, Brazil. This work presents results on evaluation of six new and two formerly obtained amphidiploids for resistance to leafspots and rust. The experiment was conducted in two years at Pindorama, experimental area of Pólo Regional Centro Norte in a complete ramdomized block design with four replications. Parent species of most of the amphidiploids, as well as two *A. hypogaea* controls were also included. No disease control was provided to the experiment. Diseases were scored in each plot in a 1 to 9 scale at three dates along the growing cycle. The data from the three evaluations were expressed in area under the disease progress curve. All amphidiploids, except (*A. ipaënsis* x *A. duranensis*)^{4x} for late leaf spot, showed higher resistance than the *A. hypogaea* controls. Amphidiploids kept, as a whole and for the three diseases evaluated, the same resistance level of their respective wild parental species. Considering the three diseases, (*A. magna* x *A. cardenasii*)^{4x} and (*A. magna* x *A. stenosperma*)^{4x} showed outstanding behavior, as compared to the other amphidiploids.

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Gene-expression profile of five runner peanut genotypes response to short-term water deficit utilizing controlled rainout shelters

Notes

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Drought can significantly limit peanut productivity, depending on duration and/or severity. Under a mild or short term stress, plants have the ability to acclimate and exhibit higher tolerance to stress at a different time. Determining plant gene-expression profile in response to water deficit at different development stages will elucidate important metabolic pathways that may be involved in drought resistance or tolerance. Five runner-type peanuts with varying responses to drought were grown under controlled rainout shelters and subjected to a short-term (3wk) at mid-season (43-71 days after planting). A small set of candidate transcripts were evaluated in the drought study: Early light-induced protein, Thioredoxin, Actin, HSP 70, Rubisco Activase, Cu/Zn superoxide dismutase, Late Embryogenesis Abundant protein, non-specific Lipid Transfer Protein, Tonoplast Intrinsic Protein, putative drought induce protein, metallothionein, and myo-inositol phosphate synthase. RNA was extracted from collected leaves and subjected to real-time PCR. Results showed an acclimation process and differences in peanut genotypes.

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Notes

Isolation and characterization of important genes related to embryo development

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Seed development is key to yield and quality formation in peanut (*Arachis hypogaea L.*), but the underlined mechanism of embryo development is still not known so far. We employed embryos treated with deficient/abundant calcium in soil as materials, which tend to produce sterile (bare-pods) and fertile (full-pod) embryos, respectively, to disclose early molecular events. We constructed two SSH libraries from deficient and abundant calcium-treated embryos and screened the libraries for differential expression genes in small scale by macroarray which were identified by RT-PCR. We also constructed a mixed full-length cDNA library with both treated embryos and a random chosen of 298 clones were sequenced acquiring 288 ESTs. To thoroughly screening key genes related to early embryo development, we employed high density of microchip with 101344 unigenes as probes to screen key important genes by comparative hybridization with RNA from deficient and abundant calcium-treated embryos. Three thousand one hundred and seventy-six genes with more than 2 fold up/down-regulated expression were isolated and were analyzed by Blast2GO. Twenty-nine kinases and 39 transcription factors with more than 2 folds up/down regulated expression were screened. Among them 4 kinases, calcium calmodulin-dependent protein kinase IIa, type receptor kinase, protein kinase c and a carbohydrate kinase-like protein, with over 12 folds up/down-regulated expression in deficient calcium treatment were selected for further evaluation of importance in embryo development. Their expression patterns were characterized..The obtained results offered would supply with basis for studying geocarpy and molecular mechanism of embryo development in peanut.

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Toward understanding molecular mechanism of peanut resistance to *Ralstonia solanacearum*

Notes

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Peanut bacterial wilt suffered from *Ralstonia solanacearum* infection is a serious disease in China and in Asia and the underlined mechanism is not yet discovered in all important crops including peanut. To mining genes used for reverse study and to phenotype and genotype genetic resistance gene. One full length cDNA libraries derived from mixed root inoculated with/without *R. solanacearum* was constructed for a resistant variety. A total of 1739 genes with over 2 folds of up/down regulated expression were found by microarray analysis using root RNA materials challenged with or without *R. solanacearum* with 846 genes showing upregulated expression and 893 genes showing down-regulated expression. Among them 7 NBS-LRR genes were isolated, which were characterized for their expression pattern and further studies are carried in the lab by reverse genetics methods to identify their function related to resistance. We also developed a mapping populations derived from Yueyou 92 × Xinhuishiaoli, with Xinhuishiaoli high susceptible to N. solanacearum but high resistant to *A. flavus* and Yueyou 92 high resistance to N. solanacearum but not resistant to *A. flavus,* which produced 1200 and 1000 F_2 generations, respectively, and have been advanced by single seed descendant to the F_5/F_6 generation this spring and eventually to F_7/F_8 as RIL next year. The F_2 generation was phenotyped as a ratio of 3 susceptible to one resistance when inoculated with *N. solanacearum* and were genotyped by X^2 test as one core gene inheritance. The obtained results may lead to understanding underlined mechanism.

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Notes

The haplotypes of *Arachis correntina* and *A. villosa* reflects an associated history with the alluvial fan of the Parana River during the Upper Quaternary

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Arachis correntina and *A. villosa* are perennial diploid species ($2n=2x=20$). The former is found in the Northeast Argentina and East of Paraguay River near Asunción (Paraguay), while the latter growths in a disjunct area along Uruguay River, from 29ºS to the North bank of La Plata River. Both taxa are morphologically similar and they have been considered as only one species. They show the most southern distribution of the section and most of their populations are associated to watercourses. In order to understand the colonizing pathways and to shed light on the evolutionary relationships between *A. correntina* and *A. villosa* we analyzed the haplotypic variability in the whole range of distribution of these species. For this purpose, a Neighbour-Joining tree was constructed from two cpDNA regions (*trn*T-S and *trn*T-Y) isolated from 28 populations. Six haplotypes were identified. One of them was shared by all *A.villosa* and some *A. correntina* accessions and it was widely distributed at the edges of the Paraná megafan and along the Uruguay River. The other five were exclusive of *A. correntina*, and in general, they were restricted to smaller areas associated to present rivers that are thought as ancient paleochannels. The network analysis of haplotypes revealed a close relationship between both species. The mutational pathways of haplotypes evidenced a more ancient dispersion of the haplotype shared by *A. correntina* and *A. villosa* and a more recent dispersion of those exclusive for *A. correntina* through the main river channels of the alluvial megafan generated by the Paraná River during the Upper Quaternary.

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Peanut breeding program for drought resistance/ tolerance

Notes

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The peanut (*Arachis hypogaea* L.) is a globally valued crop for its protein and oil content and quality. Currently, Argentina has become the world's leading exporter in an international scenario that forecasts a growing demand. Faced with the prospect of expansion of the Argentinean peanut planted area and considering the water limitations of its territory, 63% arid and semiarid, this program aims at characterizing and evaluating the drought resistance / tolerance of 50 genotypes from the Nursery El Carmen available germplasm collection, to develop new cultivars. Field trial started in the 2008-09 growing season and continued in 2009-10. The variables selected as indicators of water stress resistance / tolerance were: a) leaf malondialdehyde content, b) leaf chlorophyll concentration, c) harvest index, and d) pod yield. These variables were recorded for the initially selected 50 genotypes in a field trial put through induced water stress. They were also recorded in replicated plots under irrigation used as controls. Subsequently, we calculated the differences between the values obtained in normal conditions minus the values registered under the induced water stress conditions, which were subjected to principal components analysis and biplot construction, cluster analysis and minimum span tree analysis. The observed associations between variables and genotypes allowed distinguishing several clusters. These groupings discriminate six genotypes of El Carmen, among cultivars and advanced selection lines, which will continue under evaluation along with introduced varieties characterized as drought resistant / tolerant controls.

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Notes

New studies on *Arachis batizocoi* as a B-genome donor for introgression of wild alleles into cultivated peanut

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Arachis batizocoi is a wild species that for some time was considered a possible ancestor and B genome donor to cultivated peanut. However, subsequently this possibility has been discarded based on cytogenetic data and molecular markers. Most recently it has even been suggested that the genome of *A. batizocoi* should be classified as a K-type genome, related to, but distinct from, the B-genome. In spite of this, the empirical observation remains that *A. batizocoi* hybridizes easily with other species, and also that it did effectively act as a B-genome donor in the best characterized event of wild introgression in cultivated peanut to date, the introgression of root-knot nematode resistance. In this work the ability of *A. batizocoi* to act as a B-genome donor was further investigated. By controlled crosses and treatment with colchicine, 31 synthetic tetraploids were produced from the cross *A. batizocoi* K9484 x *A. stenosperma* V10309, 23 from *A. batizocoi* K9484 x *A. duranensis* V14167 and 12 from *A. batizocoi* K9484 x *A. duranensis* SeSn2848. These synthetic tetraploids were found to be fertile. Furthermore, cytogenetic analysis revealed that they harbor the expected complements of chromosomes. Furthermore they produced fertile hybrids with five different cultivars of peanut, and showed high resistance to rust. It can be concluded that although the genome of *A. batizocoi* is phylogenetically diverged from the B-genome of cultivated peanut it is an effective B-genome donor and shows good potential for use in hybridization schemes to introduce wild genes into cultivated peanut using the tetraploid route.

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New synthetic tetraploids for the introgression of wild alleles into cultivated peanut

Notes

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The transfer of genes from wild *Arachis* species to cultivated peanut is highly attractive as a way to broaden the genetic base of peanut crop. This strategy has achieved some notable successes, but its wider adoption has been hindered by some fundamental problems. These problems include fertility barriers caused by species incompatibilities, and linkage drag of desirable wild alleles with ones that confer agronomically unadapted traits. Together, these problems are considerable, but as the knowledge base improves, the ability to overcome these problems should also improve. Research over the last few years has provided a much better understanding of the origin of cultivated peanut and the relationships of the species that are closely related to its A and B genome components. Recently several studies using cytogenetics and molecular markers have indicated that two species may have potential for introgression, being distinct from, but closely related to the ancestors of cultivated peanut. These species are the A-genome *A. villosa,* and the B-genome *A. gregoryi.* Here we describe the production of two new synthetic tetraploids using these species, (*A. ipaensis* x *A. villosa*)^{4x} and (*A. gregoryi* x *A. stenosperma*)^{4x}. We also show that both new synthetics are disease resistant (especially the latter) and that they are crossable with cultivated peanut.

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Funding: FAP-DF, Generation Challenge Programme TL1, Host Institutions

Notes

Large scale transcriptome analysis of wild peanut (*Arachis stenosperma*) inoculated with *Passalora personata*, the causal agent of Late Leaf Spot

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Peanut (*Arachis hypogaea*) is one of the most important oilseed in the world due to its high energy value and nutrition. However, peanut has a low genetic variability, especially for agronomical traits, which differs from its wild species, which are sources of disease resistance and adaptation to various environments. *Arachis stenosperma* is a wild species resistant to some pests and diseases, among them, the Late Leaf Spot, caused by the fungus *Passalora personata*. Aiming to identify genes associated with response to resistance, the transcriptome of *A. stenosperma* V10309, challenged with the fungus *P. personata* and its control was analyzed by large scale pyrosequencing (http://www.454.com – Roche). For this, leaves of plants inoculated with $5x10^5$ spores/ml were collected 24, 48 and 72 hours after inoculation. A pool of total RNA comprising equal amounts of the three collection points was formed for cDNA synthesis and construction of two cDNA libraries (Creator SMART cDNA library construction kit, Clontech).The libraries were sequenced by pyrosequencing454 technology (GS-FLX Titanium Fragment Series Kits, Roche Applied Science) resulting in 194.076 reads from the infected sample and 168.555 from the control, covering respectively 51.609.348bp and 48.784.925bp. After analysis by bioinformatics, 39,620unigenes were identified and primers were designed for validation of candidate genes by quantitative real-time PCR. This dataset will be an important tool for identifying differentially expressed genes, characterization of wild alleles and development of molecular markers for peanut.

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A transcriptional approach for identifying genes related to drought stress

Notes

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Stress-responsive mechanisms triggered in plants under drought stress affect plant growth and cause serious limitation to crop productivity. Due to its high genetic diversity and adaptation to a range of environments, wild relatives of peanut constitute a rich source of allele diversity for resistance to biotic and abiotic stresses. Based on previous data and as a first step to identify drought-responsive genes in *Arachis*, the wild specie *A. magna*, accession KG30097, was selected as it showed high adaptability to water stress conditions. The transcriptome of *A. magna* leaves submitted to gradual water stress was analyzed. Subtractive libraries were constructed with cDNA from leaf tissues of stressed and well-watered control plants. Subtractive hybridization was performed in both directions: cDNA from stressed plants was used as driver and afterwards as tester, allowing for the enrichment of genes either induced or inhibited during water stress. *In silico* analysis revealed 759 reads, which were grouped into 249 clusters, with a novelty index of 32,8%. Several up and downregulated genes were identified exclusively in stressed or control conditions. The expression profile of some differentially regulated genes was validated by real time PCR, using cDNA from roots and leafs of stressed and control plants. Glycine descarboxilase, metallothionein-like protein, drought stress responsive protein, and two unknown proteins were shown to be up-regulated and the gene coding for a disease responsive protein was down-regulated. The information produced in this study is a valuable resource for gene identification, characterization of new wild alleles, and development of molecular markers.

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Notes

BAC clone selection using a 3D pooling strategy in *Arachis*

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Bacterial Artificial Chromosome (BAC) libraries are fundamental tools for genomic studies, important for physical mapping, map-based gene cloning and analysis of gene structure. Easy handling and propagation of clones, relative stability and low degree of chimerism have made BAC vectors the cloning system of choice. Polymerase chain reaction (PCR)-based screening of BAC pools is attractive because it is simple, fast and sensitive. It also has the advantage of avoiding radioactive labeling. We describe the use of a simple system for the preparation of three-dimensional pools of an *Arachis duranensis* BAC library and identification of clones of interest. The 219 plates (384-well) of the entire library were divided into 20 pooling units (PU), each containing 11 plates. For each PU, BAC pools were generated in three-dimensional matrices. Each well in the stack has a unique address defined by its x (column number in a plate), y (row number in a plate), and z (plate number) coordinates. For each PU, three copies of the stack plates were prepared for three-dimensional pooling. After growth, we collected for each PU 24 pools in the x dimension, 16 in the y and 11 in the z, totaling 51 pools that were extracted by alkaline lysis. This procedure was accomplished in three months for the whole library, using only one employee and no handling robotics. PCR using primersfor genes evolved in biosynthesis of peanut oil and ESTs related to disease resistance and drought tolerance will be used to select clones from the pools in order to characterize and map them.

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Transcriptome analysis of *Arachis stenosperma* for identification of resistance genes to *Meloidogyne arenaria*

Notes

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Root-knot nematodes are a group of endoparasites with a large range of host plants. Juveniles penetrate root tip epidermis and form feeding structures called giant cells. The infection leads to a transcriptional reprogramming, resulting in the formation of galls. *Arachis hypogaea* is parasitized by *Meloidogyne* species that cause significant yield losses. Resistance to *M. arenaria* was indentified in *A. stenosperma* as a hypersensitive-like response that prevents giant cell formation. Aiming to understand the early molecular response of *A. stenosperma* to *M. arenaria* and identify genes involved in its resistance, massal transcriptome analysis of infected roots was performed. The bioassay was carried with plants inoculated with juveniles of *M. arenaria*. Root samples were collected at time points: 0, 3, 6 and 9 days after inoculation. The sequence assay was performed in a HiSeq2000 Illumina System. cDNAs libraries, consisting of two biological replicates, were constructed for each time point including adaptors for the multiplex sequencing. It was produced 114,979,230 reads, showing an equal distribution between libraries. The error rates, measure by PhiX reference genome, were below 2% (1.31 and 1.47%), numbers in accordance to Illumina specifications for 100 bp reads. The percentages of reads having a base quality greater or equal than Q30 were 82.7 and 81.7%, higher than 70%, the recommendation for reads longer than 75 bases. This EST databank will allow a better evaluation of the gene expression involved in *Arachis* resistance to *M. arenaria* and can be used as a basic resource for molecular marker development and gene discovery.

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Notes

Reference genes for RT-qPCR expression studies in wild and cultivated *Arachis* species

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Plant transcriptome analysis under specific experimental conditions helps the understanding of cellular processes related to development, stress response, and crop yield. The validation of these studies has been accomplished by RT-qPCR which requires normalization of mRNA levels among samples. This can be achieved by comparing the expression ratio between a gene of interest and a reference gene which is constitutively expressed. Nowadays there is a lack of appropriate reference genes for wild and cultivated *Arachis*. In the present work, a simplified RT-qPCR protocol based on SYBR reagent was used for the identification of genes with minimal expression variation in *Arachis*. Ten reference genes were analyzed in four *Arachis* species (*A. magna*, *A. duranensis*, *A. stenosperma*, and *A. hypogaea*) subjected to biotic (root-knot nematode and leaf spot fungus) and abiotic (drought) stresses, in two distinct plant organs (roots and leaves). By the use of three algorithms (GeNorm, NormFinder and BestKeeper), five genes (ACT1, UBI1, GAPDH, 60S and UBI2), emerged as top reference genes in eight samples. The former three were the most stable across all species, organs and treatments studied. To ratify the expression stability of candidate reference genes, the expression profile of an *A. magna* gene induced by water deficit was analyzed using reference genes (60S and UBI2) selected in this study. The use of the appropriate reference genes characterized here should improve the accuracy and reliability of gene expression analysis in peanut and other legumes and contribute for the better understanding of gene expression.

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Differential gene expression study of wild *Arachis* under a water deficit induction system

Notes

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Wild species of *Arachis* are very diverse genetically and were selected, throughout the course of evolution to adapt to diverse environments. Therefore, the wild species can offer ample resources to resistances to biotic and abiotic stresses. In particular, the wild species, *A. duranensis* and *A. stenosperma*, have a high adaptability to water stress conditions, and were chosen for the present study. The objective of this work was to identify genes of those wild species that are expressed in response to gradual drought stress. *A. duranensis* and *A. stenosperma* seedlings grown in nutrient Hewitt's solution hydroponically were submitted to water stress separated in 3 blocks that constitute the biological replicates. Plantlets were placed in wood supports in such a way that the roots were completely immersed in the solution and were maintained in a greenhouse. After 15 days, plantlets were submitted to different treatments in which they were removed from the hydroponic solution and kept in a tray in the dark without nutrient solution for 0 min (control), 25, 50, 75, 100, 125 and 150 min (3 to 4 plantlets per treatment). Roots and leaves were collected at each treatment point and immediately frozen in liquid nitrogen. RNAs were extracted by the lithium chloride method of and treated with DNAse, providing high quality material. cDNAs libraries will be constructed for pools of time points, in biological replicates, and sequencing will be performed in a HiSeq2000 Illumina System.

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Financial support: CNPq, FAP/DF, Generation Challenge Program and Host Institutions.

Notes

Large scale transcriptome analysis of wild peanut (*Arachis duranensis*) under gradual water stress

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Cultivated peanut, *Arachis hypogaea,* is one of the most widely grown grain legumes in the world due to its high energy value. However, most of domesticated species are susceptible to biotic and abiotic stresses, resulting in production losses. Unlike, wild species are sources of alleles related to disease resistance and adaptation to different environments, which are desirable traits of economic importance. In particular, the wild species, *A. duranensis* (access K7988), has a high adaptability to water stress conditions. The aim of this study is to identify gene expression regulation in *A. duranensis* plants subjected to gradual water stress and its control irrigated plants. Leaves and roots were collected at five different points of stress, when changes were observed in the pattern of plant transpiration, and after 30 minutes and 72 hours of rehydration. Total RNA was extracted and a pool was formed for each treatment, using equal amounts of total RNA from individuals in each collection point and for each tissue. Two cDNA libraries were constructed from total RNA purified from these pools and sequenced by pyrosequencing large scale technology 454 (GS-FLX Titanium Fragment Series Kits, Roche Applied Science). A total of 380,601 reads (average size of 390pb) was generated and 21,710 Unigenes were obtained after clustering and assembly. The most differentially expressed candidate genes related to abiotic stress were selected for validation through RT-qPCR and their relative RNA expression patterns determined. All information generated here will be important for the characterization of new wild alleles, gene discovery and development of molecular markers.

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Peanut gene expression induced by infection of *Ralstonia solanacearum*

Notes

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To elucidate the mechanism of resistance to bacterial wilt (BW) caused by *Ralstonia solanacearum* in peanut, molecular response to the bacterial infection was investigated using resistant peanut line Zh3103 via sequencing of expressed sequence tags (ESTs). The results indicated that gene expression in both leaves and roots was greatly influenced by *R.solanacearum* infection. Of the total 5622 leaf and 7371 root unigenes, 2750 and 2730 genes in leaf and root respectively were observed only after infection of the pathogen. The expression patterns in peanut leaf and root were similar, indicating that these categories of genes might be related to pathogenesis or responsible for BW resistance. Genes related to post-translational modification and translation, ribosomal structure, cell and cell part, cellular process, and metabolic process might play an important role in cellular response to the bacterial infection. Many differentially expressed genes were ethylene responsive transcription factors or were involved in ethylene signal transduction pathway. Out of 51 genes encoding putative ethylene responsive transcription factors, 12 were up-regulated either in leave or root. Other components of ethylene signal transduction pathway such as *etr1*, *MAPK*, and *MAPKKK* were also significantly enhanced after pathogen infection, indicating that ethylene signal transduction pathway might be involved in the pathogenesis. Realtime PCR analysis of some specific genes such as *PR-10*, *Lectin*, and genes encoding ascorbate peroxidase, methionine and lipid transfer protein as well as those ethylene responsive genes were significantly enhanced by *R.solanacearum* infection, which was consistent with the data obtained by EST sequencing.

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Notes

Identification of candidate genes in *Arachis stenosperma* involved in the interaction with root-knot nematode (*Meloidogyne arenaria*)

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Root-knot nematode (*Meloidogyne arenaria*) infection represents a limiting factor for peanut (*Arachis hypogaea*) production. The cultivated peanut species has a narrow genetic base and some wild *Arachis* species could be an alternative source of nematode resistance, showing hypersensitive-like defense response and resistance to root-knot nematode. In the present study, *A. stenosperma* (accession V10309) roots challenged by *M. arenaria* are used to identify candidates genes involved in its resistant interaction. Bioassay was carried out with roots inoculated with juveniles of *M. arenaria* and samples were collected at time points: 0, 3, 6 and 9 days after inoculation (approx. 10 plants per point). Total RNA from roots was extracted using Trizol Reagente® and two pools (5 plants per pool) were formed for each collecting point. cDNA was synthesized from each pool and samples treated with DNAse. Leg 066 primers were used to check DNA contamination by RT-PCR. Primers were then designed from eight host candidate genes, previously identified by our group as involved in the resistant responses of peanut to nematode challenge. RT-qPCR was performed with those primers to determine their efficiency. Two genes (Auxin Repressed Protein and Cytokinin Oxidase) were further selected for validation through RT-qPCR and their relative RNA expression patterns analyzed. Both genes showed distinct expression profiles in *A. stenosperma* roots during its resistance response to *M. arenaria*. The study of the expression profile of host genes is an important step to understanding the mechanisms involved in peanut-nematode interaction.

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Screening of groundnut (*Arachis hypogaea* L.) minicore collection for mutant allele of Oleoyl-PC desaturase to identify high oleic accessions

Notes

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Oil stability and quality in groundnut largely depends on proportion of MUFA (oleic acid) and PUFA (linoleic acid) present and their ratios. Loss of function of oleoyl-PC desaturase activity with mutant alleles ah*FAD2A* and ah*FAD2B* results in high oleate trait in groundnut. Minicore collection and checks (196) were screened for reported mutant alleles at ah*FAD2A* and ah*FAD2B* by employing CAPS markers (Chu *et al.*, 2007). Majority of the accessions with mutant *ahFAD2A* allele belonged to ssp.*hypgaea* Virginia Runner (88.23%) and Virginia Bunch (85.71%) ,whereas, majority of the accessions of ssp. *fastigiata* had wild type allele. However, few accessions of ssp.*fastigiata* had mutant *ahFAD2A* allele, the frequency of which was more in Valencia (30.55%) compared to Spanish Bunch (19.50%) accessions. This allelic (ahFAD2A) survey in mini core collection supported the earlier view that ssp.*fastigita* was primitive of the two subspecies from which ssp.*hypogaea* originated through mutation The accessions with distinct oleic acid content were also screened to detect the reported mutant allele of *ahFAD2B* with CAPS marker without any success even in the high oleic (74%)accession probably because of some other mutation different from the reported A insertion in coding region for which CAPS marker was developed. It was also evident by *per se* performance of accessions that, genotypes with wild type allele had 40-45% oleic acid and those with more than 50 per cent oleic acid had *ahFAD2A* mutant allele which could be further increased with presence of another mutant allele of *ahFAD2B* . One of the ssp.*hypogaea* accession, ICG 2381 with high oleic acid (74%) was identified that had mutant allele of *ahFAD2A* but mutant allele *ahFAD2B* could not be detected with CAPS marker developed for detection of insertion mutation. This investigation led to the grouping of the accessions based on *ahFAD2A* allele variation and identification of new sources for high oleic acid that help in broadening the genetic base of new cultivars with high oleate trait.

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Notes

Genetic enhancement of nutritional quality traits with induced mutagenesis in groundnut (*Arachis hypogaea* L.)

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Groundnut (*Arachis hypogaea* L.) kernels contain high quality edible oil (45%) and easily digestible protein (24%). However, the limited accessible genetic variability for these quality traits restricts the genetic improvement of the crop. An attempt made to induce genetic variability through physical (gamma-rays) and chemical (EMS) mutagenesis with two Spanish bunch cultivars (TPG41 & GPBD4) resulted in significant increase in genetic variability for nutritional quality traits at Main Agricultural Research Station of University of Agricultural Sciences, Dharwad.The protein content of kernels has been enhanced from 23.8% to >28% in three mutants of TPG41 and from 30.75 to > 34% in seven mutants of GPBD4. Oil content has been increased from 45.7% to >48% in three mutants of TPG41 and marginal increase of 1% in GPBD4 from 48.8% to 49.8%. However, there has been significant enhancement in oil quality with increase in oleic acid from 50.3% to > 60% in seven mutants of GPBD4 and from 59.8% to >67% in four mutants of TPG41 that resulted in increased O/L ratio from 1.66 to >3.3 in five GPBD4 mutants and from 2.82 to > 4.75 in four TPG1 mutants. The significant increase in seed size was also achieved from 37.4g to > 47g/100 seeds in seven mutants of GPBD4 and from 63g to> 72g/100 seeds in four mutants of TPG 41 These new mutants isolated for the above quality traits could add to the genetic resources kit of the crop for further genetic improvement to mitigate malnutrition and health problems in semi – arid tropics of Asia where groundnut crop is predominantly grown for edible oil and protein.

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Behavior of *Arachis hypogaea* breeding lines under infestation of *Enneothrips flavens* and *Stegasta bosquella*

Notes

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Thrips (*Enneothrips flavens*) and Redneck worm (*Stegasta bosquella*) are major pests of the peanut crop in southeastern Brazil. Insecticide applications are needed to prevent plant damages and yield reduction. Differences between cultivars have been reported regarding insect infestation and responses to chemical control, suggesting the interest of evaluating larger number of genotypes starting with the germplasm pool of *A. hypogaea*. Thirty five advanced breeding lines of IAC, previously selected for resistance to foliar diseases, were evaluated in a field experiment at Pindorama, SP in a split plot design with complete randomized blocks and three replications. The main plots consisted of controlling and not controlling the insects, and the subplots comprised the breeding lines and five cultivars. Insect presence was noted in "no insect control" plots at seven dates along the growing cycle, in twenty leaflets randomly collected from each plot. Pod yield was evaluated after harvesting, and the percentage of yield reduction between controlled and not controlled treatments was estimated. Average number of thrips/10 leaflets along all dates of evaluation ranged from 3.24 (line IAC 318) to 7.37 (line IAC 368). The number of Redneck worms/10 leaflets ranged from 1.90 (lines IAC 333 and 335) to 8,57 (line IAC 371). Percentage of yield reduction in not controlled plots (due to both insects) ranged from 0.0 to 43.3%, and a number of lines showed low percentages. Further experiments are planned to confirm these data, as well as to investigate possible resistance/tolerance mechanisms.

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Notes

Methodology adaptation for the determination of resveratrol in peanut leaves

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Resveratrol, an antioxidant compound associated with human diseases prevention, has been detected in many peanut tissues. Our objective was to test a previous reported methodology to evaluate resveratrol in wild *Arachis* species leaves. The resveratrol synthesis was induced using UV light. Dried (40ºC for 44 h) and fresh material were extracted with 80% ethanol in a polytron® (20.000rpm for 2 min) and then centrifuged. Since interfering was observed in the two extracts (dried and fresh) both were purified using hexane partition extraction and alumina column purification. The purified extracts were dried at 60ºC for 30 min, dissolved in 15% ethanol and then centrifuged. Hexane partition extraction was better than alumina column purification since it had a 97% recovery of resveratrol, with repeatability below 5%. Despite the background was observed that made necessary an extra purification with ethyl acetate. The resulting extract was analyzed by high performance liquid chromatography (HPLC). No significant differences were observed between the dried and fresh samples. It was also observed that heating of sample during extraction and extraction preparation did not interfered in resveratrol stability. Ethyl acetate extraction promoted an efficient purification. This method had a linearity between 0.01 and 217.6 µg/mL ($r^2$0,9998) and injection repeatability of 1.18 (at 23µg/mL), showing the method is efficient to detect resveratrol in *Arachis* leaves.

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Determination of resveratrol in leaves of wild *Arachis* species

Notes

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The compound resveratrol is a plant secondary metabolite produced when plant is under biotic or abiotic stresses. The objective of this study was to quantify levels of resveratrol in ten wild *Arachis* species. UV treated leaves of each species were analyzed 15 hours after 2:30 hours of UV treatment. Each sample was analyzed in triplicate. One gram of leaves was macerated in liquid nitrogen. The extraction was performed on tissue homogenizer (Polytron®) for 2 min at 20.000 rpm with 12 mL of 80% ethanol. Two mL of the supernatant extract were purified by partition with hexane and dried on hot plate and nitrogen gas for 35-40 min at 60 °C. The dried extract was dissolved in 0,7 mL of 15% ethanol and then centrifuged at 14.650 rpm for 17min. The supernatant was injected into HPLC. The extract was always protected from light to avoid isomerization. *Arachis hypogaea* had in average 510µg/g for induced samples and 7.37 µg/g for control; *A. kuhlmannii* 860,9µg/g and 39,9µg/g; *A. duranensis* 670,3µg/g and 9,3µg/g; *A. cardenasii* 601,6µg/g and 26,5µg/g; *A. batizocoi* 551,5µg/g and 8,8µg/g; *A. magna* 390,9µg/g and 26,6µg/g; *A. cruziana* 386,6µg/g and 4,3µg/g; *A. kempff-mercadoi* 341,9µg/g and 4,2µg/g; *A. gregoryii* 231,1µg/g and 3,4µg/g; *A. ipaënsis* 220,2µg/g and 12,3µg/g and *A. simpsonii* 198,3µg/g and 10,4µg/g. All species showed resveratrol and the level in non induced and induced samples varied a lot being *A. kuhlmannii* the species that had the highest content of resveratrol for both control sample and induced sample.

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Notes

Development of genetic linkage maps for the A and B genomes of *Arachis* using RIL populations

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Cultivated peanut is an allotetraploid (AABB) with limited genetic diversity. Wild *Arachis* species represent a rich source of useful genes and traits. Exploitation of genetic variation of wild species can be facilitated by the use of molecular markers and genetic maps. We have been working to provide an improved genetic framework for peanut, making diploid and tetraploid maps using a common set of markers. The tetraploid genetics of peanut was dissected by constructing one diploid map for the A genome and one for the B genome, including the most probable wild ancestors of peanut as parents. The AA map was constructed using an F_5 population derived from an *A. duranensis* x *A. stenosperma* cross and it has 71 microsatellite, 79 anchor markers, 332 SNP, and 17 RGA markers. The BB map (*A. ipaënsis* x *A. magna*), based on an F_6 population, has 132 SSR markers, many in common to the AA map. A tetraploid map is underway, based on an F_6 population obtained through the crossing of a cultivated peanut cultivar (Runner IAC 886) with a synthetic amphidiploid (*A. ipäensis* x *A. duranensis*)^{4x}. Overall the markers' order is highly conserved between maps. Since the maps were based on RIL populations, phenotyping assays are being conducted for several traits in different environments. In this way we aim to map consistent QTLs in different genetic backgrounds, and move towards marker-assisted selection in breeding programs to efficiently introgress wild species alleles into cultivated peanut.

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SSR-based genetic diversity analysis of *Arachis hypogaea* accessions

Notes

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The knowledge of the genetic variability of peanut (*A. hypogaea* L.) accessions is important for their efficient conservation and use in breeding programs. In the present study, 34 fluorescent-labeled microsatellite markers were used for the analysis of the genetic relationships among 145 accessions of cultivated peanut and two accessions of *A. monticola*. *Arachis hypogaea* accessions were collected in different geographic regions of South America and represent the two subspecies (*fastigiata* and *hypogaea*) and their six botanical varieties (*fastigiata*, *vulgaris*, *aequatoriana*, *peruviana*, *hypogaea* and *hirsuta*). We also included accessions collected in Xingu Indigenous Park, which have morphological traits, especially of the pods, that exceed the described variation in cultivated peanut. Pairwise genetic similarities were estimated using the band-sharing coefficient of Lynch, 1990. The resulting matrix was then submitted to cluster analysis using UPGMA. Six main groups were evident, clustering accessions of the same varieties, with some exceptions. These results corroborated the current taxonomic classification, except for the varieties *peruviana* and *aequatoriana*, which were closer related to the accessions of the subspecies *hypogaea* than to the other two varieties of subspecies *fastigiata*. The Xingu samples formed a differentiated group close to the variety *hypogaea*. Considerable levels of genetic variability were detected in the Brazilian Germplasm Collection. The identification of similarity groups, jointly with morphologic and agronomic data, will be useful for the selection of parental plants to be used in the peanut breeding programs and for the construction of genetic maps.

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Notes

Analysis of the A-TR2 variability and chromosome localization in different species of *Arachis* section

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Section *Arachis* has 29 wild diploid and two allotetraploid species belonging to five different genomes (A, B, D, F and K). Based on molecular mapping and GISH analysis it was proposed that changes in the repetitive fractions may have been a main force leading to genomic differentiation in *Arachis*. To test this hypothesis, we focused on the tandemly repeated fraction, particularly in a sequence previously isolated from the centromeric heterochromatin of *A.duranensis,* A-TR2. We isolated and characterized 67 sequences from peanut and from diploid species representative of all the genomes of the section and, also, from the three different karyotype groups recognized within the A genome. The sequence was highly conserved with only 14 variable sites. None of the A-TR2 variants was species specific; however, the polymorphism detected within each species tends to be largely distinct from those of other taxa. FISH analysis detected hybridization of A-TR2 only on chromosomes of the species belonging to the three A-genome karyotype groups and of the F-genome. However, in none of these species we observed hybridization onto all the heterochromatic bands, but each species was particular in the number A-TR2 signals. These results suggest a differential composition of the centromeric heterochromatin present in the chromosomes within a single species. The bulk of data evidences that A-TR2, although present in all the taxa analyzed, has different variants, representation and probably organization among chromosomes, karyotype groups and genomes, suggesting that it was actively involved in the genomic differentiation of *Arachis* species.

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Notes

A-TR2, a satellite sequence of the centromeric heterochromatin of *A. duranensis*

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Highly repetitive DNA sequences are main components of eukaryotes genomes. Different types of these sequences may coexist within a particular genome and it was proposed that changes in their composition and representation actively participate in the genomic differentiation of plants. Sections *Arachis* comprise 29 wild diploid species and two allopolyploid (*A. hypogaea* n.v. peanut and *A. monticola*) which were arranged in five different genomes (A, B, D, F y K). Recent analysis of molecular markers suggested an extensive colinearity among some of these genomes, while GISH experiments suggest a marked difference in the repetitive component of them. For this reason, we focused on the repetitive fraction of *Arachis* species, particularly in the tandemly repeated sequences. In this work, we isolated and characterized a repeated sequence, A-TR2, present in the genomes of *A. duranensis*. The length of the basic repeated units ranges from 308 to 324 bp, and form tandem arrays in 91 GSS of the public databases. The detailed analysis of these sequences revealed a high content of AT bases (82 to 84 %) and the presence of several motifs involved in the dispersion and chromatin packaging mechanisms of tandemly repeated sequences. Moreover, the results obtained by FISH indicate that this sequence is one of the main components of the centromeric C-DAPI$^+$ blocks in at least five chromosome pairs of *A. duranensis*. Thus, the data on nucleotide composition, organization and chromosomal distribution evidence that A-TR2 is a typical centromeric satellite DNA sequence, the first described for *Arachis* genomes.

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Notes

Carbon isotope discrimination of representative Brazilian cultivars under drought stress

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Peanut production in Brazil is changing rapidly from the cultivation of traditional Valencia type varieties, represented by the cultivar BR-1 (developed by Embrapa for the Northeast region) to the Virginia runner types, represented by the cultivar IAC Runner 886 (developed by the Campina Agricultural Institute, for the Southeast region). Developed in contrasting environments, these cultivars were derived from rigid selection for reproductive characteristics. Higher yielding US peanut cultivars produced highest yields than many drought tolerant genotypes in experiments under water stress and under fully irrigated conditions, unexpected results for drought traits evaluations. Carbon isotope discrimination has been used for estimating effective use of water (EUW). BR-1 and Runner 886 plants were individually cultivated in soil-filled pots (50 cm height and 20 cm diameter), under drought stress and well watered conditions, in a randomized design with five replications. Pots were watered daily until reach field soil water capacity. At the beginning of flowering stage for each cultivar, water supply was suspended for drought treatment pots replications. Leaf Water Potential (LWP) was measured until reach -3.0 MPa, in predawn (12th DAIS); plants were carefully harvested. The first expanded leaf was collected at each replication to evaluate Leaf Carbon Isotope Discrimination (LCID). Under well-watered and drought stress, BR-1 showed highest LCID values than Runner 886, indicating that BR-1 maintains higher levels of stomatal conductance in both conditions. These results indicate that LCID can be useful to estimate EUW, especially useful for large genotype screening.

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Financial support: Embrapa

Introgression of resistances for rust and late leaf spot in peanut from wild species using the tetraploid route through backcross

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Peanut (*Arachis hypogaea*) is a legume of great importance for human nutrition. Amongst the greatest yield reducers are the leaf spots and rust. Wild species have been largely studied due to their high potential as sources of genes that confer resistances for the improvement of the culture. As an effort to introgress wild genes into the cultigen, a BC_1F_2 population was generated using as parentals (*Arachis hypogaea* cv. IAC-Runner-886 and the amphidiploid (*A. ipaënsis* KG30076 x *A. duranensis* V14167)^{4x}. Fourty families were field evaluated. All hybrids were confirmed using SSR markers. Twenty-four BC_1F_3 families were selected based on evaluations for resistance to late leaf spot, arquitecture, productivity and nodulation. Further selection was done and 12 families of BC_1F_4 were planted in the following year. When compared to the cultivated parental, a considerable number of lines exhibited superior resistance to leaf diseases and nodulation, with similar arquitecture and levels of productivity. These results show a surprisingly rapid recovery of domestication traits in *Arachis* through back-crossing and selection and emphasise the potential of synthetic materials for improving peanut.

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The effect of tetraploidization of wild *Arachis* on leaf anatomy and other drought related traits

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Peanut is widely cultivated in the tropics, where unpredicted drought stress is a limiting factor for production. Conversely, wild species are found in extremely diverse environments, therefore, it seems likely that wild species harbor genes that could confer improved abiotic tolerance to the peanut crop. The response of cultivated peanut to drought stress has been widely investigated, but not to the wild relatives. In order to a more cost-effective choice of species/accessions for crosses to obtain new amphidiploids, we investigated the anatomy and physiological responses relevant to drought in two wild relatives of peanut: A. duranensis and *A. ipaensis*, and the amphidiploid (*A. ipaensis* x *A duranensis*)^{4x}, compared to the cultivated *A. hypogaea* cv. Runner IAC-886. We also investigated how the induced tetraploidization related to bridging wild genes into the cultigen causes some morphologic changes. This study alerts on: How far direct evaluations of wild diploid species can be used to identify desirable traits that may be used to improve the drought tolerance of cultivated peanut? The parameters used were anatomical and physiological. Some parameters are inherited from one of the parents, whereas others change upon tetraploidization. The wilds did not show obvious advantageous features when compared to the cultigens, therefore their adaptation to dry environment may be due to mechanisms of escape of avoidance. The results here suggest that screening for wilds in the diploid stage has limited used for breeding, purposes: phenotyping is likely to be more meaningful on plants at the tetraploid stage.

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