**AAGB 2015 Abstracts**

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**Table of Contents**

**Page**

**Analysis of the diploid and tetraploid *Arachis* genomes**

Scott A. Jackson, Dongying Gao, David J. Bertioli, Soraya Leal Bertioli, Robert Schmitz,

Peggy Ozias-Akins ….………………………………………………………………………………………………**1**

**Recombination between the A- and B-subgenomes has generated genome diversity in cultivated peanut**

David Bertioli, Brian Abernathy, Soraya Bertioli, Kenta Shirasawa, Scott A. Jackson …………………………..…**1**

**Next generation genomics, genetics and breeding in peanut**

Rajeev K. Varshney ………………………………………………………………………………………..…………**2**

**Gene expression profiling in cultivated peanut: Putative gene functions and candidate gene discovery**

Peggy Ozias-Akins, Josh Clevenger, Ye Chu, Larissa Guimaraes, Thiago Maia, Wei Huang,

Mary Duke, Brian Scheffler, Steven Cannon ...............................................................................................................**2**

**Development of high density 60K “Axiom\_*Arachis*” SNP Chip and optimization of genomic selection**

**model for enhancing breeding efficiency in peanut**

Manish K. Pandey, Gaurav Agarwal*,* Abhishek Rathore, Pasupuleti Janila, Hari D. Upadhyaya,

Josh Clevenger, Scott Jackson, Xuanqiang Liang, Peggy Ozias-Akins, Rajeev K. Varshney …………………….....**3**

**Identification of large-scale SNPs for the development of a 60 K SNP array in groundnut**

Josh Clevenger, Carolina Chavarro, Brian Abernathy, Gaurav Agarwal, Manish Pandey,

David Bertioli, Scott Jackson, Rajeev Varshney, Peggy Ozias-Akins …………………………………………….......**3**

**Using PeanutBase: Features, examples, and tips**

Steven Cannon, Wei Huang, Ethalinda K.S. Cannon, Sudhansu Dash, Scott Kalberer,

Longhui Ren, Nathan Weeks, Julie Dickerson, Pooja E. Umale, Andrew Farmer ………………..…………………**4**

**Transcriptome analysis of a peanut seed coat mutant and its wild type reveals expression**

**coordination of ligin and flavonoid pathways in peanut seed coat development**

Liyun Wan, Yanshan Wu, Bei Li, Yong Lei, Liying Yan, Huifang Jiang, Xiaoping Ren, Yuning

Chen, Xiaojing Zhou, Li Huang, Rajeev K Varshney, Boshou Liao …………………………………………………**4**

**Identification of QTLs for use in marker assisted selection in peanut breeding**

C. Corley Holbrook, Peggy Ozias-Akins, Ye Chu, Thomas G. Isleib, Josh Clevenger,

Carolina Chavarro, Scott Jackson, Albert Culbreath, Tim Brenneman, Charles Chen,

Christopher Butts, Marshall Lamb, Tom Sinclair, Avat Shekoofa, Barry Tillman,

Mark Burow, Baozhu Guo ………………………………………………………..………………………………….**5**

**Differential expression during seed and pod biogenesis through RNA-Seq analysis**

Carolina Chavarro, Peggy Ozias-Akins, Scott A. Jackson, Brian Abernathy……………………………………… ...**5**

**RNAi-mediated control of aflatoxins: Method to assess its effectiveness in peanut, and workflow**

**to study genetic diversity of aflatoxigenic *Aspergillus***

Renée S. Arias, Paola C. Faustinelli, Victor S. Sobolev…………………………………………………………. …..**6**

**Identification and utilization for resistance to aflatoxin in peanut**

Boshou Liao, Yong Lei, Huifang Jiang, Liying Yan, Xiaoping Ren, Liyun Wan,

Yuning Chen, Xiaojing Zhou, Houmaio Wang, Li Huang ……………………………………………………..……**6**

**Use of genomics for breeding for tolerance to water deficit stress in peanut**

M.D. Burow, M.G. Selvaraj, J. Chagoya, J.L. Ayers, V. Belamkar, R. Chopra, P. Sankara, B. Zagrè

M'biBertin, C. Corley Holbrook…………………………………………………………………………………. …...**7**

**MABC and MAS enabled breeding of early maturing peanuts with high oleic trait and resistance**

**to diseases**

P. Janila, Rajeev K. Varshney, Manish K. Pandey, Murali T. Variath, Yaduru Shasidhar,

Surendra S. Manohar, T. Radhakrishnan, N. Manivannan, K. L. Dobariya, R. Vasanthi,

Bera S.K., Manish K. Vishwakarma, H.L. Nadaf, N. Premalatha ……………………………………………………**7**

**Association mapping of SSR markers to leaf spot and TSWV resistances in cultivated peanut**

**RNA-sequencing to understand mechanisms of drought stress acclimation response in peanut roots**

Y.Y.Tang, C.Y.Chen, P.M. Dand, A. Hagan, K. Bowen, G. He……………………………………………………. ..**8**

**RNA-Sequencing to understand mechanisms of drought stress acclimation response in peanut roots**

Kameswara Rao Kottapalli, Sandhiya Arun, Pratibha Kottapalli, Diane Rowland, Paxton Payton ………………….**8**

**Transcriptome analysis of *Aspergillus flavus* reveals isolate specific gene profiles in the response**

**to oxidative stresses and carbon sources *in vitro***

Jake C. Fountain, Spurthi N. Nayak, Manish Pandey, Vinay Kumar, Prasad Bajaj,

Ashwin S. Jayale, Anu Chitikineni, Liming Yang, Brian T. Scully, R. Dewey Lee,

Robert C. Kemerait, Rajeev K. Varshney, Baozhu Guo ……………………………………………………………..**9**

**Molecular analysis of rosette resistance in groundnut crosses by reversed transcriptase**

**polymerase chain reaction**

A. Usman, S.K. Offei, Danquah E. Ofori, S.G. Ado, M.F. Ishiyaku, C.A. Echekwu…………...……………........... **9**

**Hi-Oleic peanuts improve biomarkers of cognitive, vascular and cardiometabolic health in**

**middle aged adults**

A.M. Coates, J.A. Barbour, J.D. Buckley, J. Bryan, P.R.C. Howe ….…………………………………………..……**9**

**Phylogenetic relationship of peanut germplasm as revealed by tGBS**

Xinyou Zhang, Bingyan Huang, Feiyan Qi, Lijuan Miao, Lei Shi, Wenzhao Dong, Fengshou Tang ………..……..**11**

**Evaluating chloroplast markers for *Arachis* phylogeny at low taxonomic levels and DNA barcoding**

João Lucas Dilly, Lorena Ramos da Mata, Marcio de Carvalho Moretzsohn, José Francisco M. Valls…………… **11**

**Breeding of high oleic, early maturing peanut varieties for the Australian peanut industry**

**Part 1: Breeding strategy and genetic gain**

G.C. Wright, D. Fleischfresser,L. Owens, A. Cruickshank, D. O’Connor ……..…………………………….……**12**

**Physiological analysis of yield improvement of ultra-early peanuts in variable rainfed**

**production environments of Australia**

Yashvir Chauhan, R.C.N. Rachaputi, Steven Krosch, Graeme Wright ………………………………..………….**13**

**Peanut varieties for coastal areas of Andhra Pradesh, India**

Dr. V.S.G. Rao Sunnam ……………………………………………………………………………………………**13**

**The success story of Kadiri 6: A high yielding early maturing groundnut variety suitable**

**for semiarid regions of India**

K. Raja Reddy, K.S.S. Naik, A. Prasanna Rajesh, B. Santosh Kumar Naik, K.Vemana,

E. Chandrayudu, C. Prathyusha ……………………………………………………………………………………...**14**

**K1454 Red: A high yielding, high oil, early maturing, multiple resistant, Virginia bunch**

**groundnut variety developed for semi-arid tracts of India**

K.S.S. Naik, A. Prasanna Rajesh, B. Santosh Kumar Naik, K. Raja Reddy, K. Vemana,

E. Chandrayudu, C. Prathyusha ……………………………………………………………………………………..**14**

**Opportunities for marker assisted selection in the University of Florida peanut breeding program**

B.L.Tillman………....………………………………………………………………………………………… …….**15**

**Effect of weather parameters on development and progress of late leaf spot (*Phaeoisariopsis personata*) disease in groundnut**

Anil Pappachan, R. Sarada Jayalakshmi Devi, Shreeshail Sonyal …………………………………………….…....**15**

**Genetic resources: Where do we go from here?**

H. T. Stalker……………………………………………………………………………...………………………..... **16**

**SNPs discovery and fluidigm genotyping in a cultivated peanut x wild species F2 population**

Carolina Ballén-Taborda, Soraya Leal-Bertioli, Joe Morrissey, Don Livingston, Ye Chu,

Corley Holbrook, Peggy Ozias-Akins, Scott A. Jackson, David Bertioli ...……………………………………..….**16**

**Mapping late leaf spot and rust resistance using an improved consensus map in**

**peanut (*Arachis hypogaea* L.)**

R.S. Bhat, R.M. Kolekar, B. Asha, M. Sukruth, K. Shirasawa, V. Sujay, Y. Khedikar, C. Sarvamangala,

M.V.C. Gowda, B.N. Motagi, R.K. Varshney ………………………………………………………….……….**17**

**Evaluation of multiple stress tolerant groundnut genotypes for productivity and**

**nutritional quality in Nigeria**

B.N. Motagi, H.A. Ajeigbe, C. Echekwu, S.G. Mohammed, A.A. Adnan, L.O. Omoigui, H.M Desmae,

E. Monyo, P. Okori, P.Janila, H.D. Upadhyaya, R.K. Varshney, R. Tabo ………………………………………......**17**

**Multiple biotic stress resistant and productive genotypes identified under Spanish**

**bunch background in groundnut (*Arachis hypogaea* L.)**

B.N. Motagi, M.V.C Gowda, G.K. Naidu, H.L. Nadaf, R.S. Bhat, P.V. Kenchangoudar …...........................….......**18**

**Evaluation of groundnut genotypes for resistance to *Sclerotium rolfsii* under artificial**

**field inoculated conditions**

S. Pujer, P.V. Kenchangoudar, M.V.C. Gowda, B.N. Motagi…………………………………………………… …**18**

**Cloning and functional analysis of peanut *SAD* promoter**

Lei Shi, Fei-yan Qi, Suo-yi Han, Bing-yan Huang, Wen-zhao Dong, Feng-shou Tang, Xin-you Zhang…..…….. ..**19**

**Use of SNP technology for marker-assisted breeding using peanut interspecific**

**introgression lines**

Mark D. Burow, Ratan Chopra, Roshan Kulkarni, Theophilus Tengey, Jennifer Chagoya,

Jeffrey Wilson, Michael R. Baring, A. Hillhouse, Charles E. Simpson …….………………………………….…....**19**

**CRISPR/Cas9-mediated genome editing in peanut**

Mei Yuan, Phat Dang, Charles Chen, C.S. Prakash, Guohao He …………………………………………………....**20**

**Redox systems are a potential link between drought stress susceptibility and the exacerbation**

**of aflatoxin contamination in crops**

Liming Yang, Jake C. Fountain, Xinzhi Ni, Pingsheng Ji, Brian T. Scully, Robert C. Kemerait,

Robert D. Lee, and Baozhu Guo…………………………………………………………………………… ……….**20**

**Construction of a SNP-based genetic linkage map by ddRADseq and QTL detection for**

**resistance to late leaf spot and plant type-related traits in peanut**

Xiaojing Zhou, Youlin Xia, Xiaoping Ren, Yulin Chen, Li Huang, Boshou Liao,

Yong Lei, Liyin Yan, Huifang Jiang ……………………………………………………………………………......**21**

**Deep sequencing-based comparative transcriptional profiles for response to aflatoxin production**

**by *Aspergillus flavus* in resistant and susceptible peanut genotypes**

Houmiao Wang, Yong Lei, Liyun Wan, Liying Yan, Xiaoping Ren, Yuning Chen,

Xiaojing Zhou, Huifang Jiang, Boshou Liao……….…………………………………………………….............. …**21**

**Integration of rapid phenotyping and genotyping tools for peanut genetic improvement**

D.J. O’Connor, R.C.N. Rachaputi, R.J. Henry, A. Furtado ………………………………………………………....**22**

**Evaluation of intensity and duration of seed dormancy in a recombinant inbred population**

**derived from Spanish bunch genotypes**

Y.B. Naganagoudar, P.V. Kenchangoudar, B.N. Motagi, M.V.C. Gowda, H.L. Nadaf, S. Pujer ………………..…**22**

**Response of groundnut mini core collection to iron deficiency chlorosis**

Omprakash Kumar Singh, S.K. Pattanashetti, B.D. Biradar, G.K. Naidu,

H.D. Upadhyaya, M.K. Pandey, R.K. Varshney………………...……………………………………………. ….…**23**

**Phenotyping of a RILs population derived from a synthetic amphidiploid for peanut**

**Smut Resistance**

F. de Blas, S. Soave, L. Torres, C. Oddino, M. Pepermans, J. Soave, M. Buteler ………………….…………. …..**23**

**Screening of groundnut interspecific derivatives for resistance to *Sclerotium rolfsii***

M. Balaraju, P.V. Kenchangoudar, B.N. Motagi, S.S. Adiver, M.V.C. Gowda, S. Pujer………....…………… …...**24**

**Molecular cloning and characterization of phospholipase D from peanut (*Arachis hypogaea*)**

Silong Chen, Boshou Liao, Yurong Li …………………………………………………………………………….**24**

**Chromosome structural stability but canalized amphiplasty in AABB allotetraploids of *Arachis***

G. Seijo,L. Chalup, S. Samoluk, A.P. Fávero and G. Robledo ……………………………………………………...**25**

**Genetic mapping of microsatellite markers based on genome survey sequences and**

**expressed sequence tags in *Arachis* species**

Li Huang, Bei Wu, Jiaojiao Zhao, Xiaoping Ren, Yuning Chen, Xiaojing Zhou, Boshou Liao,

Huifang Jiang …………….…………………………………………………………………………………………**25**

**Combining biotech and conventional methods to develop high-oleic, Sclerotina blight resistant**

**peanut cultivars**

Kelly Chamberlin, Ning Wang, Rebecca Bennett, John Damicone ...........................................................................**26**

**Development of novel SSR makers within resistance gene analogues for groundnut**

**(*Arachis hypogaea* L.)**

Y. Amaravathi, R.P. Vasanthi, K. Raja Reddy ……………………………………………………………………..**26**

**Genotype and environment influence on antioxidant expression and antioxidant related**

**proteins in *Arachis hypogaea***

Yan Yee Poon, Sridevi Muralidharan, Graeme Wright, Paul Haynes, Alice Lee …………………………………...**27**

**Host range of the peanut root rot pathogen *Fusarium neocosmosporiellum***

Kyle Wenham…………………………………………………………………………………………………….... ..**27**

**Author Index** ………………………………………………………………………………………………………**28**

**AAGB Conference**

**List of Abstracts**

**Analysis of the diploid and tetraploid *Arachis* genomes**

Scott A. Jackson\*, Dongying Gao, David J. Bertioli, Soraya Leal Bertioli,

Robert Schmitz, Peggy Ozias-Akins

University of Georgia, USA

\*sjackson@uga.edu

With the completion of the first drafts of the diploid peanut progenitors, *Arachis ipaensis* and *A. duranensis*, we now have unprecedented opportunities to explore the genome structures and changes that occurred in the formation of the tetraploid, cultivated peanut, *A. hypogea*.  We have annotated the repeated DNAs, primarily transposable elements (TEs) where we found 1,900 transposon sequences that composed more than 69% of the peanut genomes, more than any other sequenced legume. We also see instances of mobilization of elements in the tetraploid from either parental donor genome. Since they are ‘silenced’ via an epigenetic pathway that includes DNA methylation, we have also examined the methylation of these genomes and found extensive methylation of repeated structures as well as methylation in genes, the type of which often correlates with how genes are expressed (regulated).  Together with the draft genomes, we are beginning to gain insights into the structure of the *Arachis* genomes as well as functional characterization of how the genome is organized and genes are regulated.

**Recombination between the A- and B-subgenomes has generated genome diversity in cultivated peanut**

David Bertioli1,2\*, Brian Abernathy1, Soraya Bertioli1,3, Kenta Shirasawa4, Scott A. Jackson1

1University of Georgia, Georgia, USA; 2University of Brasília, Brasília, Brazil; 3EMBRAPA, Brasília, Brazil; 4Kazusa DNA Research Institute, Kisarazu, China, Japan.

\*djbertioli@gmail.com

Cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid that arose less than 10,000 years ago from a hybridization event between two diploid species, the A-genome donor *A. duranensis* Krapov. & W.C. Greg. and the B-genome donor *A. ipaënsis* Krapov. & W.C. Greg. Here we report the use of the genome sequences of these diploid ancestors to investigate the genome structure of *A. hypogaea*. We used the diploid chromosomal pseudomolecules as “scaffolds” onto which sequence reads of cultivated peanut can be overlaid or “mapped”. For the most part, the cultivated peanut genome closely approximates the addition of the two diploid genomes, and has a genome composition that can be expressed as “AABB”. However, some genome regions in the tetraploid have suffered deletions, meaning that the genome composition is best expressed as “AA--”, “--BB”, or even “----”. In other regions there has been autotetraploid-like tetrasomic recombination between the A- and B-subgenomes resulting in genome compositions that can best be expressed as “AAAA” or “BBBB”. Furthermore, especially for the A-subgenome, gene-conversion events with the B-subgenome have occurred. These deletion and recombination events have occurred in somewhat different ways in different cultivated peanut genotypes. We suggest that recombination between the A- and B-subgenomes provides a diversifying force for the evolution of the peanut crop.

**Next generation genomics, genetics and breeding in peanut**

Rajeev K. Varshney

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India

[r.k.varshney@cgiar.org](mailto:r.k.varshney@cgiar.org)

Next generation genomics approaches such as second- and third- generation sequencing and high-throughput genotyping technologies are bringing paradigm shift in genetics and breeding approaches in many crops including peanut. With the availability of genome sequence for diploid progenitors and tetraploid genome sequence due in coming months, in conjunction with high-throughput phenotyping platforms and appropriate decision support tools, accelerated development of superior peanut varieties by deploying genomics-assisted breeding is expected. For instance, by using genome re-sequencing or transcriptome sequencing of several accessions of tetraploid as well as diploid species, one 60 K “Axiom\_*Arachis*” SNP chip has been developed. Next generation mapping populations such as multi-parent advanced generation intercross (MAGIC) and nested-association mapping (NAM) populations are being developed for undertaking high-resolution mapping. So far with the available genetic and genomic resources, limited success has been achieved in trait mapping using either bi-parental populations or germplasm sets. Although molecular markers are already available for rust resistance, late leaf spot resistance, root-knot nematode resistance and high oleate trait and they are being used in molecular breeding in routine, complex traits such as yield under drought stress are yet to be addressed. In this context, a novel breeding approach called, ‘genomic selection (GS)’ is being deployed by using “Axiom\_*Arachis*” SNP chip and appropriate GS models. In addition, new functional genomics approaches such as transcriptomics, proteomics, and metabolomics have also been initiated for enhancing understanding of complex traits. Recent advances with future prospects on above mentioned approaches, we have made at ICRISAT in collaboration with our partners, will be presented in the meeting.

**Gene expression profiling in cultivated peanut: Putative gene functions**

**and candidate gene discovery**

Peggy Ozias-Akins1\*, Josh Clevenger1, Ye Chu1, Larissa Guimaraes1, Thiago Maia1,

Wei Huang2, Mary Duke3, Brian Scheffler3, Steven Cannon4

1Institute of Plant Breeding, Genetics & Genomics, University of Georgia Tifton Campus, Tifton,

GA 31793, USA; 2Dept. of Agronomy, Iowa State University, Ames, IA, 50011, USA; 3USDA ARS JWDSRC, 141 Experiment Station Rd., Stoneville, MS  38776-0038; 4USDA-ARS Corn Insects and Crop Genetics Research Unit, Crop Genome Informatics Lab, Iowa State University, Ames, IA, 50011, USA

\*pozias@uga.edu

A gene expression atlas for cultivated peanut is under development that is based on transcriptional profiling using RNA-Seq of vegetative and reproductive tissues of the reference genome genotype, ‘Tifrunner’.  Differential expression across organs and tissues can be viewed in the genome browsers at peanutbase.org and information can be used to develop and test hypotheses on gene function. Alternative splicing events have been identified in three categories, exon skipping, alternative donor, and alternative acceptor.  Tissues responding to pest or pathogen infection also have been sampled to expand gene expression studies beyond normal developmental states of leaf, shoot apex, root, root nodules, flower structures, gynophores and pods (pericarp and seed).  Sequencing libraries from roots of four genotypes challenged with root-knot nematode (*Meloidogyne arenaria*), leaves of two genotypes challenged with late leaf spot pathogen (*Cercosporidium personatum*), and pods of six genotypes challenged with *Aspergillus flavus* have been constructed.  The four genotypes exposed to root-knot nematode included resistant and susceptible parents and resistant and partially resistant recombinant inbred lines.  In addition to identifying groups of genes responsive in only resistant or susceptible lines, sequences also were analyzed for single nucleotide polymorphisms, which allowed delineation of recombination breakpoints in the two recombinants and identification of a candidate nematode resistance gene.  These data are enabling the discovery of novel alleles and gene expression patterns, which inform marker-assisted selection and candidate gene identification in peanut, thereby enhancing breeding tools for cultivar improvement.

**Development of high density 60K “Axiom\_*Arachis*” SNP Chip and optimization of**

**genomic selection model for enhancing breeding efficiency in peanut**

Manish K. Pandey1, Gaurav Agarwal1*,* Abhishek Rathore1, Pasupuleti Janila1, Hari D. Upadhyaya1, Josh Clevenger2, Scott Jackson2, Xuanqiang Liang3,Peggy Ozias-Akins2, Rajeev K. Varshney1\*

1International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India; 2University of Georgia (UGA), USA; 3Crops Research Institute, Guangdong Academy of Agricultural Sciences (GAAS), China

*\**[r.k.varshney@cgiar.org](mailto:r.k.varshney@cgiar.org)

Molecular breeding has already delivered products for simple traits such as foliar fungal diseases (rust and late leaf spot resistance), root-knot nematode resistance and high oleate trait in peanut using marker-assisted backcrossing (MABC) approach. However for improving complex traits, genomic selection (GS) has emerged as the most promising approach that captures small-effect QTLs and develop superior lines with multiple traits. In this context, a training population (TP) comprising of 310 elite genotypes has been constituted and is being phenotyped for several agronomically important traits. In order to increase efficiency of GS breeding, we have developed a high density 60K “Axiom\_*Arachis*” SNP Chip which will be used to generate high throughput genotyping data on TP and subsequent breeding generations (and also for accelerating high-resolution mapping). To develop above mentioned chip, initially a total of 163,782 SNPs were identified including 113,735 SNPs (58, 438 SNPs for A-genome and 55,397 SNPs for B-genome) from WGRS data of 23 tetraploid species accessions, 49,947 SNPs (39,937 SNPs for A-genome and 10,010 SNPs for B-genome) from WGRS data of 7 accessions of diploid species and 5,025 SNPs for B-genome from RNA-seq data of 3 accessions of tetraploid species. Finally, a total of 58,000 SNPs were selected which have genome specificity and are highly informative. Meanwhile, the ‘minicore collection’ (184 genotypes) was genotyped with 15,360 diversity array technology (DArT) features and phenotyped for days to flowering, seed weight and pod yield. Upon testing six GS models on the phenotypic and genotypic data on the ‘minicore collection’, Ridge Regression-BLUP and Bayesian LASSO were identified as best performing GS models based on high cross-validation values. Updates on progress on GS and development and applications of “Axiom\_*Arachis*” SNP Chip will be presented in the meeting.

**Identification of large-scale SNPs for the development of a 60 K SNP array in groundnut**

Josh Clevenger\*, Carolina Chavarro, Brian Abernathy, Gaurav Agarwal, Manish Pandey,

David Bertioli, Scott Jackson, Rajeev Varshney, Peggy Ozias-Akins

University of Georgia, USA

[\*jclev@uga.edu](mailto:*jclev@uga.edu)

*Arachis* genomics has lagged behind other crops in discovery of single nucleotide polymorphism (SNP) markers, the most ubiquitous, useful, and powerful markers for genomics.  An international collaboration, led by ICRISAT, The Peanut Genome Consortium, and the University of Georgia, has developed an Affymetrix 60K SNP Chip including SNPs from all botanical types of *Arachis hypogaea* and six wild diploid *Arachis* species.  Using the tool, SWEEP, and diploid genome sequences as reference, over a million high quality SNPs were identified from 23 allotetraploid *A. hypogaea* accessions. An additional million SNPs were identified between A genome species *A. cardenasii*, *A. stenosperma*, and three *A. duranensis* accessions, and between B and K genome species *A. magna, A. ipaënsis,* and *A. batizocoi*. We filtered using the following set of criteria: (1) low copy in the genome (2) SNP localization within a predicted gene or within 4 kb of a predicted gene and (3) sufficient sequence available to reconstruct the cultivated sequence with the alternate base.  We included 15,752 SNPs from the diploid species and a set of 25 validated SNPs associated with Rust, Late Leaf Spot resistance, and high oleic fatty acid ratio.  This SNP Chip will be a community resource that will open the door for new genomics advances in *Arachis*, including genomic selection, association mapping, and high density QTL mapping.  These studies will provide identification of tightly linked markers to traits of interest and will facilitate more powerful marker-assisted breeding.  The 60K chip will bring about the new age of peanut genomics.

**Transcriptome analysis of a peanut seed coat mutant and its wild type reveals expression coordination of ligin and flavonoid pathways in peanut seed coat development**

Liyun Wan1\*, Yanshan Wu1, Bei Li1, Yong Lei1, Liying Yan1, Huifang Jiang1, Xiaoping Ren1,

Yuning Chen1, Xiaojing Zhou1, Li Huang1, Rajeev K. Varshney2, Boshou Liao1

1Oil Crops Research Institute of Chinese Academy of Agricultural Sciences

Wuhan, Hubei 430062, China; 2International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324, India

[\*wanliyun@caas.cn](mailto:*wanliyun@caas.cn)

Seed coat cracking adversely affects external appearance and reduces the commercial value of peanuts. Cracking of seed coat can also result in increased infection of pathogens. A peanut mutant spontaneously with seed-coat cracking and brown testa color, designated as “peanut seed-coat crack mutant (*pcsm*)”, was identified from an EMS-induced mutant population (with Zhonghua 16 being the wild type cultivar). By using RNA-Seq, we examined the seed coat transcriptome in three stages of immature seed development both in the wild type and the mutant. Phenylpropanoid, lignin and flavonoid pathway genes were highly differentially expressed in all stages, especially for DAF 40. Genome-wide comparative analysis of the transcript profiles revealed 62 differentially expressed genes (DEGs) in common among the three different stages. By analyzing the expression pattern and the sequences of the common DEGs of the three stages, we found that there were several candidate genes responsible for seed coat cracking. The data set generated in this study provided evidence for some functional genes as robust candidates responsible for peanut seed coat crack, which could be beneficial to peanut quality improvement in the future.

**Using PeanutBase: Features, examples, and tips**

Steven Cannon1\*, Wei Huang2, Ethalinda K.S. Cannon2, Sudhansu Dash3, Scott Kalberer1, Longhui Ren2, Nathan Weeks1, Julie Dickerson2, Pooja E. Umale3, Andrew Farmer3

1USDA-ARS, Ames, IA; 2Iowa State University, Ames, IA; 3National Center for Genome Resources, Santa Fe, NM, USA

\*steven.cannon@ars.usda.gov

We will describe the main features and use of the PeanutBase website (<http://peanutbase.org>), including genome browsers, genetic map viewers, sequence search tools, a database of traits and QTLs, and marker-assisted selection pages with detailed information about markers and accessions for some high-value peanut traits.  New features in 2015 include a gene expression atlas for cultivated peanut (from Peggy Ozias-Akins and collaborators), new ways to browse the diploid genome sequences and features, new tools for exploring genes and gene families, additional QTLs (for root-knot nematodes, bruchid resistance, kernel quality, and other traits), and more than a thousand images of germplasm accessions (pods, seeds, and plants), with links to the USDA GRIN germplasm database. Because PeanutBase is a resource for the peanut research community, we would also like to get your feedback about features that would be useful to you. PeanutBase was started in April 2013 at Iowa State University with funding from The Peanut Foundation and in-kind contributions from USDA-ARS.

**Identification of QTLs for use in marker assisted selection in peanut breeding**

C. Corley Holbrook1\*, Peggy Ozias-Akins2, Ye Chu2, Thomas G. Isleib3, Josh Clevenger2, Carolina

Chavarro2, Scott Jackson2, Albert Culbreath2, Tim Brenneman2, Charles Chen4, Christopher Butts1,

Marshall Lamb1, Tom Sinclair3, Avat Shekoofa3, Barry Tillman5, Mark Burow6, Baozhu Guo1.

1USDA-ARS; 2University of Georgia;  3North Carolina State University; 4Auburn University; 5University of Florida; 6Texas A&M University, USA

\*corley.holbrook@ars.usda.gov

Phenotyping of structured populations, along with molecular genotyping is needed for marker development in peanut.  This research is essential for making the peanut genome sequence useful to breeders because it will make the connection between genes, gene markers, genetic maps, and agronomic traits in peanut.  Several structured populations are available, and phenotyping efforts are ongoing.  Sixteen inbred mapping populations have been created using parents that maximize genetic diversity for practical breeding objectives.  First, two runner cultivars (Tifrunner and Florida-07) were selected as common parents because runner cultivars account for about 80% of the production in the U.S.  Second, eight unique parents were selected to supply diversity across market classes and botanical varieties and are donors of favorable alleles for enhancing drought tolerance and resistance to most important diseases of peanut in the U.S.  Several research groups have selected specific populations to phenotype for biotic and abiotic stresses.  Two of these populations have also been extensively phenotyped for seed and pod characteristics, and yield.  Data analysis has resulted in the identification of QTLs for resistance to several important diseases.  QTLs have also been identified for yield and grade characteristics.  Studies are ongoing in 2015 to confirm these results and to test the applicability of these QTLs in marker assisted selection.

**Differential expression during seed and pod biogenesis through RNA-Seq analysis**

Carolina Chavarro\*, Peggy Ozias-Akins, Scott A. Jackson, Brian Abernathy

University of Georgia, USA

\*mcch@uga.edu

Legumes and peanuts are an inexpensive source of plant proteins and edible oil and are characterized by developing their seeds in pods. Thus, both seed filling and pod filling are important yield components. Seed filling is the period when fast metabolic and morphological changes occur in the seed as a sequence of events controlled by the genotype and the environment, including cellular processes such as cell expansion and early desiccation, allowing changes in color, size and weight. Thus, our goal was to characterize changes in gene expression that accompany pod shell and seed biogenesis in peanut in the translocation process, using next-generation transcriptome sequencing and the two diploid transcriptomes (*A. duranensis* and *A. ipaensis*) as a reference. Therefore, Tifrunner and NC3033 were selected for pod filling and development research as they both have contrasting pod characteristics. Preliminary observations have shown that NC3033, a small-seeded Virginia type line, suffers from incomplete pod filling, despite the fact that is one of the most cylindrocladium black rot (CBR) resistant genotypes identified, it has low grades due to its small pods and low % meat in contrast to Tifrunner, a Runner elite type characterized by large seeds and good grade. Accordingly, early and late (R3-R7) seed and pod developmental stages including dissected tissues were analyzed at the transcriptome level using RNA-Seq data from 80 samples (2 genotypes, 10 tissues-developmental stages and 4 replicates) to identify differential expression between genotypes, developmental stages and tissues, to better understand seed and pod filling biogenesis.

**RNAi-mediated control of aflatoxins: Method to assess its effectiveness in peanut, and**

**workflow to study genetic diversity of aflatoxigenic *Aspergillus***

Renée S. Arias\*, Paola C. Faustinelli, Victor S. Sobolev

USDA-ARS-National Peanut Research Laboratory (NPRL), 1011 Forrester Dr. S.E., Dawson, GA, 39842, U.S.A.

\*renee.arias@ars.usda.gov

The common hypervariability of aflatoxin distribution in crops calls for new methodologies when assessing very small samples.  Our work on RNA interference (RNAi) technologies to control aflatoxins in peanuts entails knowledge of the fungus/aflatoxin resistance of seeds from individual plants.  We developed a test that requires analysis of only a few peanut seeds for aflatoxin content by UPLC (ultra-high performance liquid chromatography) to obtain consistent results. We demonstrated a 60-100% aflatoxin reduction in RNAi-modified seeds. The design of RNAi molecular constructs to silence aflatoxin synthesis genes requires finding suitable targets in the populations of Aspergillus at a particular geographical location.  Obtaining information of the genetic variability of aflatoxigenic Aspergillus by sequencing thousands of individual isolates is not affordable at present time.  We developed a practical approach that includes capillary-electrophoresis fingerprinting of just hundreds of isolates per location, followed by whole genome sequencing of the most abundant representatives.  We are using this workflow to study Aspergillus populations within U.S.A. and from several Sub-Sahara African countries.

**Identification and utilization for resistance to aflatoxin in peanut**

Boshou Liao\*, Yong Lei, Huifang Jiang, Liying Yan, Xiaoping Ren, Liyun Wan,

Yuning Chen, Xiaojing Zhou, Houmaio Wang, Li Huang

Oil Crops Research Institute of Chinese Academy of Agricultural Sciences

Wuhan, Hubei 430062, China

\*lboshou@hotmail.com

Aflatoxin contamination in peanut caused by *Aspergillus flavus* or *A. parasiticus* has been a serious health concern. Use of peanut cultivars with resistance to aflatoxin is crucial in any integrated management approaches for aflatoxin contamination. Through extensive screening of peanut germplasm including core or mini core collections selected in China and ICRISAT, several genotypes with considerable resistance have been identified. Genetics of resistance to aflatoxin production in peanut was investigated. QTLs for resistance to aflatoxin production were identified through both linkage and association analysis. An aflatoxin-resistant improved cultivar, Zhonghua 6, which was developed by using a resistant germplasm line named Taishan Zhenzhu, was found to possess higher content of resveratrol. Through global transcriptome profiling by RNA-seq, differentially expressed unigenes (DEGs) involved in phenylpropanoid-derived compounds biosynthetic pathway were induced to higher levels in Zhonghua 6 compared to the susceptible genotype.

**Use of genomics for breeding for tolerance to water deficit stress in peanut**

M.D. Burow1,2\*, M.G. Selvaraj1, J. Chagoya1,  J.L. Ayers1, V. Belamkar2, R. Chopra2,

P. Sankara3, B. Zagrè M'bi Bertin4, C. Corley Holbrook5

1Texas A&M AgriLife Research, Lubbock, TX 79403;  2Texas Tech University, Dept. of Plant and Soil Science, Lubbock, TX 79409; 3Université de  Ouagadougou, BP 7021, Ouagadougou 03, Burkina Faso; 4Institut de l'Environnement et de Recherches Agricoles / Centre National de la Recherche Scientifique et Technologique,  Kamboinsé, BP 476, Burkina Faso; 5USDA-ARS, 115 Coastal Way, Tifton, GA, USA

\*mburow@tamu.edu

Breeding for tolerance to water deficit stress is difficult, due to the many genes involved in stress response, environmental sensitivity of response, and intensive nature of measurements of response.  We are exploring two different approaches.  The first involves use of a RIL population (C76-16 x Florida 07) developed as part of the US Peanut Genome Initiative.  Significant differences were identified among RILs for all traits measured, namely flowering, SPAD chlorophyll content, paraheliotropism, and canopy temperature.  Several accessions were among the top 10 lines for two or three traits.  A significant correlation was observed between paraheliotropism and SPAD chlorophyll content, and a negative correlation was observed between canopy temperature and flowering.  In a second approach, the U.S. peanut minicore collection was screened over two years at two locations in West Texas.  Significant differences were found among accessions for SPAD chlorophyll, flowering, paraheliotropism, and canopy temperature, as well as plot height and width near the end of the growing season, and yield.  Pod yield demonstrated that several runner accessions performed better in field response measurements in West Texas than standard cultivars.  In Burkina Faso, Spanish accessions tended to perform the best in terms of yield.  Association mapping using SSR markers representing over 350 alleles identified markers for all traits but canopy temperature over multiple environments.  Several markers were associated with multiple traits.  We propose to use several accessions as parents to donate tolerance to water deficit stress to commercial materials, and attempt to validate the markers in segregating populations.

**MABC and MAS enabled breeding of early maturing peanuts with**

**high oleic trait and resistance to diseases**

P. Janila1\*, Rajeev K. Varshney1, Manish K. Pandey1, Murali T. Variath1, Yaduru Shasidhar1, Surendra S. Manohar1, T. Radhakrishnan2, N. Manivannan3, K. L. Dobariya4, R.Vasanthi5, S.K Bera2, Manish K. Vishwakarma1, H.L. Nadaf6, and N. Premalatha3

1International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Pathancheru, Telangana – 502324, India; 2ICAR-Directorate of Groundnut Research (DGR), Junagadh, Gujarat, India; 3Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India; 4Junagadh Agricultural University (JAU), Junagadh, Gujarat, India; 5Acharya N G Ranga Agricultural University (ANGRAU), Tirupati, Andhra Pradesh, India; 6Univesity of Agricultural Sciences (UAS), Dharwad, Karnataka, India

\*p.janila@cgiar.org

Peanut growing agro-ecologies of Africa and Asia need early maturing varieties to suit the short length of available growing period (LGP) and/or to escape end-off season drought. However, early maturing varieties should also combine other important traits to meet the needs of farmers, traders, processors and consumers. Genomic tools will allow breeders to combine several important traits into a single variety, besides optimizing time and resources, and enhancing selection efficiency. Marker assisted backcrossing (MABC) and marker assisted selection (MAS) were employed to combine disease resistance and high oleic trait with early maturing peanuts of Spanish type.   MABC was used to target a major QTL, explaining 82% PV for resistance to rust and 68% PV for resistance to late leaf spot. Multi-location testing in disease hot spots showed 39-79% higher mean pod yield, and 23-62% higher haulm yield of introgression lines (ILs) over recurrent parents. The selected IL’s had maturity duration similar to recurrent parent and were resistant to rust and LLS with a disease score same as donor parent, GPBD 4. In another program both, MABC and MAS were employed to target two ah*FAD2* mutant alleles governing high oleic trait. ‘SunOleic 95 R’, a Runner type peanut is used as donor parent. IL’s with oleic acid content varying from 62 to 83% were developed.  High oleic IL’s with high (53-58%), and low (42-50%) oil content were selected. Phenotyping was found to be important for confirmation of the selected marker homozygotes for the target traits.

**Association mapping of SSR markers to leaf spot and TSWV resistances in cultivated peanut**

Y.Y.Tang1, C.Y.Chen1\*, P.M. Dand2 A. Hagan3, K. Bowen3, G. He4

1Dept. of Crop, Soils and Environmental Sciences, Auburn University, Auburn, AL 36849; P.M. DAND, 2USDA-ARS National Peanut Research Lab, Dawson, GA 39842; 3Dept. of Plant Pathology, Auburn University, Auburn, AL 36849; 4Tuskegee University, Tuskegee, AL 36088.

\*[cyc0002@auburn.edu](mailto:cyc0002@auburn.edu)

The ongoing genome sequencing effort in peanut will result in numerous molecular markers that can be applied to mine valuable genes for peanut cultivar improvement. Association mapping based on linkage disequilibrium (LD) provides a more effective way to map trait loci since ancestral recombination events that occurred in natural populations present a potentially large number of alleles per locus to associate markers and traits. In-depth phenotyping of the diverse collection is likely to identify markers that can be employed by breeding programs to enrich the marker-traits detection. A diverse collection of 135 lines including mini-core, cultivars and advanced breeding lines was evaluated for leaf spot and TSWV in field plots for three years. A set of 192 SSR primers from peanut genetic linkage maps were utilized to genotype the population. Three markers named ‘pPGPseq2D12B’, ‘pPGSseq19B1’, and ‘TC04F12’, were confirmed to be associated with leaf spot and TSWV resistances. The marker ‘TC20B05’ can explain 15% phenotypical variation of leaf spot resistance. These markers could be applied in marker-assisted selection (MAS) for peanut cultivar improvement.

**RNA-Sequencing to understand mechanisms of drought stress**

**acclimation response in peanut roots**

Kameswara Rao Kottapalli1\*, Sandhiya Arun1, Pratibha Kottapalli1, Diane Rowland2, Paxton Payton3

1Texas Tech University, Lubbock, TX; 2University Florida, Gainesville, FL; 3USDA, Lubbock, TX

\*rao.kottapalli@ttu.edu

In the Texas High Plains region peanut is grown under a typical center-pivot irrigation system, where plants undergo repeated cycles of water-deficit and recovery. This necessitates identification of stress tolerant peanut varieties that can adapt to these altering water-deficit cycles. Towards this goal, a relatively tolerant peanut genotype from the US mini core collection was subjected to water-deficit stress acclimation treatment. RNA-Seq was performed on 48 root tissue samples using 108 bp paired end sequencing on an Illumina HiSeq2500 sequencer. Pairwise comparisons between different time points identified 13,391 non redundant differentially expressed genes (DEGs). Functional annotation of DEGs revealed that acclimation was controlled by several interacting pathways like calcium and G-protein coupled receptor mediated signaling, regulation by WRKY and R2R3-MYB transcription factors, proline accumulation, and activity of oxygen scavengers. The phytohormone ABA acted as a central mediator in generating this root-to-shoot stress response. Interestingly, we found that methionine accumulation is important for drought-stress acclimation in peanut. Some of these genes could be utilized by peanut breeders to improve water-deficit stress acclimation in field conditions. Additionally, we will present a public resource for mining stress responsive transcripts identified in our previous studies.

**Transcriptome analysis of *Aspergillus flavus* reveals isolate specific gene profiles**

**in the response to oxidative stresses and carbon sources *in vitro***

Jake C. Fountain1,2, Spurthi N. Nayak3, Manish Pandey3, Vinay Kumar3, Prasad Bajaj3,

Ashwin S. Jayale3, Anu Chitikineni3, Liming Yang1, Brian T. Scully4,

R. Dewey Lee5, Robert C. Kemerait1, Rajeev K. Varshney3, Baozhu Guo2\*

1Department of Plant Pathology, University of Georgia, Tifton, GA, USA; 2USDA-ARS Crop Protection and Management Research Unit, Tifton, GA, USA; 3International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India; 4USDA-ARS U.S. Horticultural Research Laboratory, Ft. Pierce, FL, USA; 5Department of Crop and Soil Sciences, University of Georgia, Tifton, GA, USA

\*bozhu.guo@ars.usda.gov

Aflatoxin contamination of peanut and maize is exacerbated by drought stress. Reactive oxygen species (ROS) are produced in host plants during drought/heat stress, and are hypothesized to stimulate aflatoxin production. In order to better understand why *Aspergillus flavus* produces aflatoxin and the role of aflatoxin in environmental oxidative stress responses, we examined the gene expression profiles of different *A. flavus* isolates (three toxigenic and three atoxgenic) under H2O2-derived oxidative stress in aflatoxin-conducive yeast extract sucrose (YES) and non-conducive yeast extract peptone (YEP) media. In total, 287.6 GB of data was generated with an average of 40.3x106 reads per sample using an Illumina HiSeq 2500 platform. Preliminary data analyses suggested that medium carbon source had the greatest effect on overall gene expression profiles likely due to altered carbon metabolic processes, developmental processes, and aflatoxin production. Based on gene expression clustering analysis, it was found that isolates which produced higher levels of aflatoxin and survived higher levels of H2O2 tended to exhibit fewer differentially expressed genes in response to increasing levels of stress than the other isolates, indicating a less vigorous response to H2O2-induced oxidative stress. In addition, mechanisms related to iron metabolism and other secondary metabolites were regulated along with antioxidant enzymes in response to increasing stress. This indicates that secondary metabolism and micronutrient availability also play important roles in oxidative stress responses in *A. flavus*. Continuing comparative analyses will examine the specific roles of aflatoxin in stress responses, and we hypothesize that ROS-mediated mechanisms potentially involved in crosstalk between stress signaling pathways and between host plants and the fungus will be revealed.

**Molecular analysis of rosette resistance in groundnut crosses by**

**reversed transcriptase polymerase chain reaction**

A. Usman 1\*, S.K Offei 2, Danquah E. Ofori2, S.G. Ado1, M.F. Ishiyaku1, C.A. Echekwu1

1Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria; 2West Africa Center for Crop Improvement, School of Agriculture and Consumer Science, Department of Crop Science, University of Ghana, Legon

\*[usmanohinoyi@gmail.com](mailto:usmanohinoyi@gmail.com)

Groundnut rosette disease (GRD) caused by a complex of three agents,  groundnut rosette assistor virus (GRAV), groundnut rosette virus (GRV), and the satellite RNA (sat RNA) of GRV  is one of the major factors limiting production of groundnut (*Arachis hypogaea)* in sub-Saharan Africa. A vector aphid, even though acquires GRAV, GRV, and sat RNA, does not always transmit the three agents together into the inoculated plant, resulting in separation of groundnut rosette disease agents in time and space. Low concentrations of the rosette disease agents in host tissues make it essential to develop a reliable and sensitive method for their detection. The objective of this study was to demonstrate the potential of RT-PCR for the confirmation of phenotypically resistant groundnut genotypes.  Sixteen groundnut genotypes with field resistance to GRD were tested against any of the three agents of GRD using one step RT-PCR. The molecular diagnosis clearly demonstrated that none of the genotypes revealed resistance to all the three components based on RT – PCR assay. Genotypes; ICGX-SM-000/20/5/P4/P1, ICIAR -19-BT and ICGV07899 that showed  negative response to GRAV, could be exploited in breeding programmes to restrict the spread of groundnut rosette disease. The GRV resistant genotypes ICG-IS-07899 X SAMNUT14 and ICIAR-19-BT X MANIPENTA could be used as sources of resistance to GRV and for commercial production under favorable conditions. The two groups of genotypes could offer a good source for molecular gene pyramiding into a single genotype to achieve broad-based genetic resistance for developing sustainable crop management strategies against groundnut rosette.

**Hi-Oleic peanuts improve biomarkers of cognitive, vascular and**

**cardiometabolic health in middle aged adults**

A.M. Coates 1\*, J.A. Barbour 1, J.D. Buckley 1, J. Bryan 2, P.R.C. Howe 1,3

1Alliance for Research in Exercise, Nutrition and Activity, University of South Australia; Adelaide, Australia; 2Department of Psychology, Social Work and Social Policy, University of South Australia, Adelaide, Australia; 3Clinical Nutrition Research Centre, University of Newcastle. Callahan, Australia

[\*alison.coates@unisa.edu.au](mailto:*alison.coates@unisa.edu.au)

We sought to investigate the effect of daily consumption of Hi-Oleic peanuts compared with a nut free diet on cardio-metabolic risk markers, cerebrovascular function and cognitive performance. In a 24 week, randomised cross-over study, sixty one healthy participants (49% males, 65 ± 1years, BMI 31 ± 1kg/m2) consumed Hi-oleic peanuts (56g/d for women; 84g/d for men) and a nut free diet, each for 12 weeks. At baseline and at the end of each 12-week period the following were assessed: energy and nutrient composition (4-day weighed food diaries), cardiometabolic health (blood pressure, arterial elasticity, body composition and fasting plasma lipids, glucose, insulin and inflammation); cognitive performance (memory, processing speed and executive function) and cerebral vasodilator responsiveness (CVR) (transcranial doppler ultrasonography). During the peanut phase compared with the nut free phase, participants consumed more energy with the majority due to an increase in monounsaturated fat. There were no differences between dietary phases in markers of cardiometabolic health except improvements in arterial elasticity and CVR were significantly improved following peanut consumption compared with the nut free diet. Measures of short-term memory, verbal fluency and processing speed were significantly improved following the peanut diet compared with control, but other cognitive measures did not change.   The significant improvements in select measures of cognitive performance and vascular function indicate Hi-Oleic peanuts may have a role in maintaining both vascular and cognitive health which are important targets for healthy ageing.

**Phylogenetic relationship of peanut germplasm as revealed by tGBS**

Xinyou Zhang\*, Bingyan Huang, Feiyan Qi, Lijuan Miao, Lei Shi, Wenzhao Dong, Fengshou Tang

Industrial Crops Research Institute, Henan Academy of Agricultural Sciences / Henan Provincial Key Laboratory for Oil Crops Improvement / Key Laboratory of Oil Crops in Huanghuaihai Plains, Ministry of Agriculture, Zhengzhou 450002, P R China

\*haasz@126.com

DNA samples from a peanut diversity panel composed of 320 highly inbred varieties from different geographical regions were studied through tGBS. The goal was to identify SNPs in the diversity panel to construct a phylogenetic tree and to analyze the relationship among elite breeding parents and their derived varieties. The available peanut genome sequence with a size of about 2.44 Gb downloaded from peanutbase.org was used as the reference genome for this study. The DNA samples were genotyped using tunable Genotyping by Sequencing (tGBSTM) technology. After trimming low quality bases, approximately 85.5% of the trimmed reads could be aligned to a single best region of the genome. SNP calling was conducted using the best alignment for each read. On average, each SNP call in each sample was supported by 9 reads allowing confident genotyping calls. A set of 37,128 high quality SNPs was used to create a phylogenetic tree. Among the germplasm analysed those belonging to subspecies *fastigiata* (G1) were clustered together first and then those of subspecies *hypogaea* (G2) were added gradually. G1 was further clustered into four subgroups, G1a, G1b, G1c, and G1d, comprising mainly of landraces of var. *vulgaris*, overseas accessions of subspecies *fastigiata*, released varieties of var. *vulgaris* from south China, and released varieties of var. *vulgaris* from north China respectively. G2 was clustered into six subgroups, G2a, G2b, G2c, G2d, G2e, and G2f, comprising mainly of released varieties from south China, released varieties from north China, accessions of var. *hypogaea* with colored seed coat, accessions of var. *hirsute,* landraces, and overseas germplasms of var. *hypogaea*. In each subgroup, varieties were clustered closely in accordance with their agronomic traits.

**Evaluating chloroplast markers for *Arachis* phylogeny at low taxonomic levels and DNA barcoding**

João Lucas Dilly1\*, Lorena Ramos da Mata2, Marcio de Carvalho Moretzsohn³,

José Francisco M. Valls3,4

1Master’s degree student, University of Brasilia, Brasilia, DF, Brazil; 2Research Analyst, Embrapa Genetic Resources and Biotechnology, Brasilia, DF, Brazil; 3Researcher, Embrapa Genetic Resources and Biotechnology, Brasilia, DF, Brazil; 4CNPq Scholarship – Brazil

\*joao.lucas.dilly@gmail.com

Plant molecular systematics and DNA barcoding techniques lean heavily on the use of chloroplast markers, because of the relatively low evolutionary rates of chloroplast genes. Due to the lack of phylogenetic studies using chloroplast markers in *Arachis*, a DNA barcoding analysis is currently underway for some species of the Wild *Arachis* Genebank of Embrapa, Brazil, with emphasis on the relationships of the Brazilian wild species *Arachis kuhlmannii* Krapov. & W.C. Greg. To date, the screening for polymorphism involved the regions *rbcL, matK*, *psbA-trnH*; and *trnL-trnF*. Based on other Angiosperm barcoding studies, these loci have potential for resolving phylogenetic and species identification problems at the species level in the genus *Arachis*. Twelve accessions of three species (*A. kuhlmannii, A. helodes* and *A. correntina*) were included on the screening. DNA sequencing was performed in the ABI 3730 platform, and the analysis, using the softwares *ChromasPro* and *BioEdit*. The four loci screened were polymorphic; however, *rbcL, psbA-trnH* and *trnL-trnF* presented higher potential on grouping into phylogenetic trees. Such regions are usually the first ones on choosing the suitable loci for phylogenetic analyses and DNA barcoding. Further analyses will embrace around a hundred accessions of twenty *Arachis* species, using *rbcL, psbA-trnH*, *trnL-trnF* along with ITS markers. There are many questions involving the evolutionary relationships among species and vicariance and other mechanisms of speciation in the genus, which can be contrasted with the plenty barriers to gene flow and biological dispersion. Clarifying these questions will likely require adjustments in the systematic structure of *Arachis*.

**Breeding of high oleic, early maturing peanut varieties for the Australian peanut industry.**

**Part 1: Breeding strategy and genetic gain**

G.C. Wright1\*, D. Fleischfresser2,L. Owens2, A. Cruickshank D2, D. O’Connor1,3

1Peanut Company of Australia, Kingaroy, Qld, Australia 4610.; 2Queensland, Department of Agriculture and Fisheries, Kingaroy, Queensland, Australia, 4610; 3The University of Queensland, Queensland Queensland Alliance for Agriculture and Food Innovation, St Lucia, Qld, Australia, 4067.

\*gwright@pca.com.au

Droughts with unpredictable timing and intensity have high frequency (>70%) in SE Queensland peanut production regions, and severely limit pod yields and lead to high aflatoxin risk. New early maturing types that can escape severe drought stress and aflatoxin risk have been developed by the Australian peanut breeding program and can mature up to 30 days quicker compared to the traditionally grown full season Virginia/runner type varieties of 140+ days duration. Much later planting time is also possible (e.g. mid January) with these genetics, which are also finding application in irrigated sugarcane farming systems where irrigation water is limited and return per Mega-litre is a key driver of productivity. Major breeding aims have been high kernel yield and relevant quality traits for premium snack food and manufacturing markets, including high oleic oil, large kernel size, high kernel %, good blanchability and great taste. As well, high levels of soil borne and foliar disease tolerance to significantly reduce input costs for growers have been incorporated into this germplasm. A pedigree breeding strategy has been employed, with the use of a selection index approach which has enabled concurrent selection for high kernel yield, high kernel % (as a surrogate measure of early maturity) and enhanced foliar disease resistance traits. The program has released 4 early maturing varieties since 2007, including Walter (2007), Tingoora (2010), Redvale (2013) and Taabinga (proposed for release in 2016). Substantial genetic improvement has been achieved in the past 8 years, with mean kernel yield performance of the most recent release (Taabinga) being 50% greater than Walter, when averaged over 15 multi-year/location trials. Kernel size has also been significantly increased, with Taabinga averaging 51% v’s 28% jumbo kernel grade (% of kernels riding over a 25/64” screen) compared to Walter. Foliar disease tolerance has also been enhanced with Taabinga being highly resistant to late leaf spot, leaf rust and web blotch relative to the highly susceptible Walter. Overall kernel yield potential of our new early maturity lines is now highly competitive with currently grown full season maturity varieties, with Taabinga able to achieve a relative kernel yield of over 90% compared to Holt (full season runner check). This early maturing genetics offers peanut growers significant savings in input costs, including water and fungicides, and hence increased overall profitability. They also potentially offer improved yield and quality for peanut production systems in higher latitudes (e.g. Argentina, South Africa, Europe), where currently grown full season maturing genetics is often too long for maximum yield and quality performance.

**Physiological analysis of yield improvement of ultra-early peanuts in variable**

**rainfed production environments of Australia**

Yashvir Chauhan1\*, RCN Rachaputi2, Steven Krosch1 and Graeme Wright3

1Department of Agriculture, Fisheries and Forestry, Kingaroy, Queensland Australia; 2Queensland Alliance in Agriculture and Food Innovation (QAAFI), University of Queensland, Australia; 3Peanut Company of Australia, Kingaroy

[\*yash.chauhan@daf.qld.gov.au](mailto:*yash.chauhan@daf.qld.gov.au)

Peanuts traditionally grown in the dry land production region of Australia have been of 150 days types referred to as full season peanuts. However, a gradual change in the region’s climate in the last 30 years towards reduced rainfall and higher temperature have increased the frequency of terminal droughts. This has made production of full season peanuts more risky and often incurring high levels of aflatoxin contamination. The breeding of ultra-early peanuts that take up to four weeks less time to mature and thus have the ability to escape terminal drought was, therefore, considered a priority to sustain the industry.   Introgression of novel genetics by breeders to overcome these limitations has led to the development and release of Walter, Tingoora, Redvale, and more recently Taabinga ultra-early varieties. However, there has been little   agronomic and physiological research relating to these new peanut varieties.  Independent agronomic assessment of the potential pod yield of these varieties suggests that the yield improvement in ultra-early peanuts has been incremental. The pod yield potential of the more recent cultivar, Taabinga, is similar to the best full season varieties despite taking three weeks less time to mature. Agronomic studies set up to evaluate genotype x management interactions using plant population as a management factor  have shown that ultra-early peanuts respond to closer planting density of up to 15 plants per m2, both in terms of pod yield and kernel grading.  These results are consistent with the evaluations conducted within the Australian breeding program.  Physiological analysis of the observed pod yield improvement suggests that increased yield of ultra-early peanuts appears to come from increased partitioning of dry matter into pod yield, and weakening of the negative relationship between harvest index and total dry matter compared to full season peanuts. Identification of genetic changes associated with these transitions are likely to lead to  speeding up of development of ultra-early peanuts towards realizing even further gains in pod yield and

**Peanut varieties for coastal areas of Andhra Pradesh, India**

V.S.G. Rao Sunnam

Agricultural Research Station, Yellamanchili-531055, Visakhapatnam Dist., A.P., India

[\*gangadharsvs@yahoo.co.in](mailto:*gangadharsvs@yahoo.co.in)

Peanut is one of the important oil seed crops grown for oil and also kernel for table purpose.  In India it is mostly grown in the states Gujarath, Andhra Pradesh, Maharastra, Orissa and Tamilnadu.  In Andhra Pradesh peanut is mainly grown in Rayalseema where the annual rain fall is about 600mm with a relative humidity of 30%.  Besides, Rayalseema the crop is grown in coastal districts where the rain fall is 1000-1200mm with a relative humidity of 70-90%.  The performance of the varieties vary from coastal to non-coastal areas.  In view of high rainfall and humidity in coastal areas the plants tend to grow vertically resulting most of the pegs remain in the air without reaching the soil to become pods.  Therefore the yields are low.  Further in the event of rains at the time of harvest during rainy season germination in the field is the problem in non-dormant varieties.  Hence, the same variety performs differently from non-coastal areas to coastal areas.  Therefore, the varieties with dormancy and short stature (Virginia types) may give better results in coastal areas. Under these circumstances 22 cross bred lines after testing in filial generations were tested in a randomized block design with three replications during rainy season 2014 at Agricultural Research Station, Yellamanchili, Visakhapatnam Dist., Andhra Pradesh, India. The plot size adapted is 6.00 sq.mt.  Among the entries tested, YLGN 8 (K1468/K4) recorded highest pod yield of 1727 kg/ha followed by YLGN 7 (K5/ISKI2004-6) with 1513 kg/ha. These entries did not germinate at the time of harvest in the event of rains received.

**The success story of Kadiri 6: A high yielding early maturing groundnut variety suitable for semiarid  regions of India**

K. Raja Reddy, K.S.S. Naik\*, A. Prasanna Rajesh, B. Santosh Kumar Naik,

K. Vemana, E. Chandrayudu, C. Prathyusha

Agricultural Research Station, Acharya N G Rang Agricultural University, Kadiri-515591, Andhra Pradesh, India

[\*drkssnaik@yahoo.com](mailto:*drkssnaik@yahoo.com)

An early maturing, high yielding groundnut variety was developed for semiarid regions of India. Its a Spanish bunch variety derived from a cross between JL 24 X Ah 316/s. Hundred kernel weight of Kadiri 6 ranges from 40 to 55g, oil content 48 % and 75% shelling. The plant height ranges from 30 to 35 cm, maturing in 100-105 days with a synchronized maturity and cluster bearing habit. High yields, fertilizer responsiveness and attractive pod and kernel characteristics have increased its popularity. It has shown to be superior in both pod and kernel yields than JL 24 based on multi-location yield trials. The increase in pod yield was between 4% to 40% in All India Coordinated Trials, 6% to 40% over JL 24 at station level trials and the mean pod and kernel yields were 2410 and 1659 kg/ha in All India Coordinating Trials, and 2074 and 1422 kg/ha in station level trials, respectively. It has a high shelling percentage (3%) and higher (8%) hundred kernel weight than JL 24.These characteristics have increased its adoption by farmers and their participation in seed production system. Its production has spread to 50% of the groundnut area of India. Besides, it has occupied 60% of Indian groundnut exports and its value increased from Rs 1,500 (2006) to 8, 500 crore (2013). The Government has recognized the potential of Kadiri 6 and initiated measures for large scale seed multiplication through seed villages and public sector seed agencies such as APSSDC, NSC, SFCI, HACA, OILFED.

**K1454 Red: A high yielding, high oil, early maturing, multiple resistant, Virginia bunch**

**groundnut variety developed for semi-arid tracts of India**

K. S. S. Naik\*, A. Prasanna Rajesh, B. Santosh Kumar Naik, K. Raja Reddy,

K.Vemana,  E. Chandrayudu, C. Prathyusha

Agricultural Research Station, Acharya N G Rang Agricultural University, Kadiri-515591, Andhra Pradesh, India

\*drkssnaik@yahoo.com

An early maturing, high yielding Virginia bunch groundnut variety K1454 red was developed for semi-arid tracts of India. This variety was derived from a cross between Vemana X Tirupati 3. The  kernels are red with smooth and  attractive testa, oil content 50.7 % , SMK 88% and 63% shelling. The plant height ranges from 30 to 35 cm, maturing in 115-120 days with an attractive pods, synchronized maturity and cluster bearing habit. It has shown tolerance reaction to thrips, PBND (1%), PSND (4%), rust (2.7 score), early (3.0) and late leaf spots (3.0) and drought, which are most important adaptive traits for semi-arid tracts. It is  superior in both pod and kernel yields than Virginia varieties like Kadiri 2, Kadiri 3 and Spanish bunch drought resistant variety Vemana  based on multi-location yield trials. The increase in pod yield was 141%, 101% and  23% over its checks respectively  at station level trials and the pod and kernel yields were ranged from 1229 to 1929 kg/ha  with a mean of 1430 kg/ha and 750 to  1244 kg/ha  with a mean 899 kg/ha in station level trials, respectively. It gave high dry haulms yield of 1823 kg/ha in Semi Arid Tracts necessary for survival of farm cattle. It has a high root mining ability and its root can penetrate up to 1.5m depth. The kernels have appreciable time of fresh seed dormancy desirable for arresting in situ germination at maturity when caught in cyclones before harvest. These characteristics have increased its adoption by farmers.

**Opportunities for marker assisted selection in the University of Florida peanut breeding program**

B.L. Tillman

University of Florida, Gainesville, FL

\*btillman@ufl.edu

The peanut industry has invested heavily in genomics research over the past five years.  Goal number five of the PGI Strategic Plan is to “Ensure that the new genetic information can be used by plant breeders to provide an adequate supply of agronomic and high quality peanut cultivars”.  From a plant breeders perspective, marker assisted breeding (MAB) is valuable if it contributes in one or more of the following ways (not exhaustive) 1) improves the heritability for a trait, 2) reduces the cost of breeding for a trait, and 3) facilitates accumulation of desirable traits into single genotypes.  Improved heritability should reduce field testing and improve the final product.  However, field testing of potential cultivars will always be required, so the cost savings of MAB in replacing field testing should be obvious and meaningful.  Similarly, cultivars with improved characteristics should be readily demonstrable.  Since MAB is an added cost in a breeding program, cost savings must be demonstrated in other areas unless more funding is allocated for MAB.  Accumulation of multiple traits, especially disease resistance, into a single cultivar is perhaps the most appealing potential for MAB.   The presentation will describe peanut breeding efforts at the University of Florida with primary focus on quality and disease resistance traits and how MAB might or might not be the preferred method of selection.

**Effect of weather parameters on development and progress of late leaf spot**

**(*Phaeoisariopsis personata*) disease in groundnut**

Anil Pappachan, R. Sarada Jayalakshmi Devi\*, Shreeshail Sonyal

Department of Plant Pathology, S.V. Agricultural College, Tirupati,Andhra Pradesh,India

\*sarareddypogu.devi@gmail.com

Groundnut (*Arachis hypogaea* L.) is cultivated in India mainly as an oil seed crop. Late leaf spot is common wherever groundnut crop is grown. Late leaf spot and rust are the most serious diseases worldwide,  leading to 50–70% yield loss. Under favorable conditions late leaf spot caused by *Phaeoisariopsis personata* accounts for 25.3 per cent reduction in pod yield and 53.0 per cent reduction in haulm yield. Premature leaf fall due to the disease is a factor that reduces yield. Weather parameters like temperature and humidity play an important role in the development of diseases. Late leaf spot development and progress is highly influenced by weather parameters. Moreover, the information on weather parameters can be used to forecast the disease by developing prediction models which will reduce the usage of fungicides to a greater extent. A field experiment was conducted in the College farm of S.V. Agricultural College, Tirupati, AP, INDIA during *Kharif* 2012 to evaluate the effect of weather parameters (on initiation and progress of late leaf spot (*Phaeoisariopsis personata*) disease in Groundnut viz., maximum temperature, minimum temperature, morning and evening relative humidity, rainfall and sunshine hours  (Kadiri-6 variety) under three different dates of sowing (July first week, July third week and August first week).Correlation and regression analysis using IBM SPSS Statistics 20 software. showed that weather parameters like minimum temperature and morning relative humidity highly influenced the Initiation and progress of late leaf spot. Minimum temperature showed significant negative correlation while morning relative humidity showed significant positive correlation with the late leaf spot development which indicated that progress of late leaf spot epidemic was favoured by high morning relative humidity (more than 80%) and low temperature. Maximum temperature showed significant negative correlation with disease progress in the two late sown conditions (July third week and August first week) while evening relative humidity showed significant positive correlation only in second date of sowing (July third week sowing). Area Under Disease Progress Curve (AUDPC) increased and both pod yield and haulm yield (kg ha-1) decreased with delay in sowing time.

**Genetic resources: Where do we go from here?**

H. T. Stalker

North Carolina State University, Raleigh, NC

\*tom\_stalker@ncsu.edu

Large collections of cultivated and wild peanut are being preserved in multiple countries and many accessions are duplicated at more than one location.  Although some materials have few seeds or poor germination, collections are generally in good condition.  Resources are adequate to preserve the germplasm collections, not sufficient to add large genetic or breeding populations.   The pertinent question is what is in these collections? Although a significant amount of germplasm evaluation has been completed at ICRISAT and to a lesser extent at other locations, there remain large gaps in knowledge about the levels of resistance for many pathogens and insect pests, quality traits, and sources of improving yield and non-biotic resistances. Data often varies among locations, which may be due to different testing methodologies, environmental interactions or possibly races of pathogens.   Several wild species have been identified very high levels of resistance to important peanut pathogens, but much of the germplasm remains to be evaluated. Several core collections have been developed and significant efforts have been made to evaluate them, but with a very few exceptions, extrapolation of this work to the larger collection has been lacking.   Another approach has been to develop diverse populations (e.g., the “CAP” set of 16 recombinant inbred lines) to establish highly variable materials for genetic investigations derived from materials with diverse levels of resistance or variable quality traits.  Several of the RIL populations are being utilized to associate molecular markers with traits of interest.  However, the parents of these populations may or may not contain genes conditioning the greatest levels of the trait of interest, and additional populations will need to be established specifically for the individual trait of interest.  Molecular markers also will facilitate introgression of desirable genes from wild species into cultivars and help to eliminate many of the undesirable traits associated with non-cultivated species, but even with markers, sterility and poor seed production is highly problematic.  In summary, resources have been available to collect and preserve peanut germplasm, but significant investments are needed to evaluate accessions, identify useful materials, and incorporate useful genes into peanut cultivars.

**SNPs discovery and fluidigm genotyping in a cultivated peanut x wild species F2 population.**

Carolina Ballén-Taborda1, Soraya Leal-Bertioli1,2\*, Joe Morrissey3, Don Livingston3, Ye Chu2,

Corley Holbrook1, Peggy Ozias-Akins1, Scott A. Jackson1, David Bertioli1,4

1University of Georgia, Athens; 2EMBRAPA, Brasília, Brazil; 3Mars, Miami, Florida;

4University of Brasília, Brazil.

\*djbertioli@gmail.com

Rust, late leaf spot (LLS) and root-knot nematodes are important reducers of peanut (*Arachis hypogaea* L.) yield and quality and increase the production costs. Because sources of resistance against many diseases are available in the wild *Arachis* species, they are of interest to peanut breeding programs. Previously, a mapping population based on a cross between highly polymorphic wild diploid species have been used to identify candidate genome regions that control disease resistance. QTLs conferred by *A. stenosperma* V10309, for late leaf spot resistance have been found on linkage groups A02 and A04 and for root-knot nematode on linkage groups A02 and A09. Knowing this, for the confirmation of function of these chromosome segments in allotetraploid peanut, a population of 218 F2 plants was developed from a cross of *A. hypogaea* and (*A. batizocoi* K9484x *A. stenosperma* V10309)4x. These were genotyped with 576 DNA markers using Fluidigm technology. The markers were selected along the A and B genomes, but mostly concentrated in the vicinity of the QTLs. This genotyping, in combination with disease resistance phenotyping that is currently underway, will allow the function of the QTL chromosome segments to be tested. This analysis will also allow us to better understand the recombination process in *A. hypogaea* x wild species*,* which likely follows a “segmental allotetraploid” model where the recombination is partly disomic and partly tetrasomic.

**Mapping late leaf spot and rust resistance using an improved consensus**

**map in peanut (*Arachis hypogaea* L.)**

R.S. Bhat1\*, R.M. Kolekar1, B. Asha1, M. Sukruth1, K. Shirasawa2, V. Sujay1, Y. Khedikar1, C. Sarvamangala1, M.V.C. Gowda1, B.N. Motagi1, R.K. Varshney3

1College of Agriculture, University of Agricultural Sciences, Dharwad-580005, India

2Department of Frontier Research, Kazusa DNA Research Institute, China 292-0818, Japan

3Center of Excellence in Genomics (CEG), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad 502 324, India

\*bhatrs@uasd.in

Late leaf spot and rust are the major biotic stresses in peanut worldwide. An effort was made to map late leaf spot and rust resistance using the recombinant inbred line populations derived from TAG 24 × GPBD 4 and TG 26 × GPBD 4 in peanut. The new genetic maps were developed by mapping a large number of *Arachis hypogaea* transposable element (AhTE) markers in addition to the previously mapped SSR markers. A consensus map was generated based on these two independent maps, which was employed for detecting the genomic regions governing late leaf spot and rust resistance measured at three stages (70, 80 and 90 days after sowing) in 12 seasons. Details of the quantitative trait loci identified from this study will be discussed so as to use them in molecular breeding of peanut for improving late leaf spot and rust resistance.

**Evaluation of multiple stress tolerant groundnut genotypes for productivity**

**and nutritional quality in Nigeria**

B.N. Motagi1\*, H.A. Ajeigbe1, C. Echekwu2, S.G. Mohammed3, A.A. Adnan3, L.O. Omoigui4, H.M. Desmae5, E. Monyo6, P. Okori 6, P. Janila7, H.D. Upadhyaya7, R.K. Varshney7, R. Tabo5

1International Crops Research Institute for Semi-­Arid Tropics (ICRISAT), Kano, Nigeria; 2Institute of Agricultural Research (IAR), Samaru, Zaria, Nigeria; 3Bayero University of Kano (BUK), Kano, Nigeria; 4University of Agriculture, Makurdi, Nigeria; 5ICRISAT, Bamako, Mali; 6ICRISAT, Lilongwe, Malawi; 7ICRISAT, Patancheru, Telangana, India

\*b.n.motagi@cgiar.org

Groundnut plays a very important economic role for smallholder farmers in the semi-arid tropics as a major cash crop for many households; a nutritious and safe food thereby contributing to improved health of the rural population. It is rich in protein, oil and micronutrients such as iron and zinc. High iron and zinc contents are especially beneficial for women and children at risk of anemia and have proven to be genetically malleable. High oleic acid and low linoleic acid make groundnut oil ideal for storage and better human health. Evaluation of  541 advanced breeding lines along with local landraces and improved varieties for their reaction to drought, rosette and foliar diseases besides productivity parameters over two locations during 2014 main season resulted in identification of  45 promising lines with significantly superior pod yield (1304-2796 kg/ha) compared to check entries (189-1005 kg/ha). Further, these superior genotypes were evaluated for nutritional quality and in trials during 2014/15 dry season to confirm their superiority. Nutritional quality (oil, O/L ratio, protein, Fe and Zn content) analyses lead to the identification of nutritionally dense genotypes. Genotypes ICGV IS 11060, Samnut 23, ICGV 00064, ICGV 01276, ICGV IS 07827 and Kampala had high oil content (53-54%); while ICGV 07813 had high O/L ratio of 6.1 followed by ICGV IS 09992, ICGV SM 05593 and ICGV SM 06722 with 3.0 O/L ratio. Genotypes ICGV IS 07833, ICGV IS 3980, ICGV SM 08553 and ICG 5891 had high protein (30-32%), Zn (46-51 ppm) and Fe (23-34 ppm) content. These serve as ideal genetic resources to develop agronomically superior and nutritionally enhanced groundnut cultivars with multiple resistances to biotic and abiotic stresses.

**Multiple biotic stress resistant and productive genotypes identified under**

**Spanish bunch background in groundnut (*Arachis hypogaea* L.)**

B.N. Motagi1,2\*, M.V.C. Gowda1, G.K. Naidu1, H.L. Nadaf1, R.S. Bhat1, P.V. Kenchangoudar1

1Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India; 2ICRISAT, Kano, Nigeria

\*[b.n.motagi@cgiar.org](mailto:b.n.motagi@cgiar.org)

The cultivated groundnut is an important oilseed crop of the world. Several pest and diseases damage the crop and reduce groundnut yields considerably. Cultivation of resistant varieties is an ecologically sound and economically viable approach to reduce the loss due to these stresses, but their occurrence and intensity vary in space and time necessitating the use of multiple stress resistant genotypes. Several diverse groundnut germplasm were assessed for different biotic stresses under epiphytotic conditions. Most of the cultivated varieties were susceptible to different stresses. Interspecific derivatives constituted the best source of resistance to late leaf spot (LLS), rust and *Sclerotium;* while mutants were superior for late leaf spot, *Spodoptera* and bud necrosis. Pedigree of multiple stress resistant genotypes revealed contribution of wild species for resistance to many biotic stresses. Trait association studies indicated late maturing nature of resistant germplasm. Induced mutagenesis and extensive hybridization with interspecific derivatives were sought to break these undesirable associations. Several foliar disease resistant mutants and second cycle interspecific-derivatives were isolated in Spanish bunch background. Mutant (28-2) and second cycle interspecific derivative (GPBD-4) were resistant to foliar diseases with high yield potential even under foliar disease epidemic. 28-2 was also resistant to *Spodoptera*, thrips and *Aspergillus* infection besides having bold kernels.  GPBD-4 was iron absorption efficient and had O/L ratio of 1.68. They also possessed desirable agronomic features, early maturity, high partitioning and better quality. Mutant 28-2 and GPBD-4 have been registered with National Bureau of Plant Genetic Resources (NBPGR) , New Delhi with INGR numbers 98003(IC296686) and 01031 (IC296810), respectively. These cultures had stable and superior performance over popular cultivars (JL-24 and TMV-2) across years. GPBD-4 has been accepted by farmers and traders; under active seed chain and cultivation in the farmers’ fields in India. GPBD-4 has been widely employed for MABC at ICRISAT and UASD as the source of resistance to LLS and rust.

**Evaluation of groundnut genotypes for resistance to *Sclerotium rolfsii***

**under artificial field inoculated conditions**

S. Pujer1\*, P.V. Kenchangoudar1, M.V.C. Gowda1, B.N. Motagi1,2

1Oilseeds Scheme, Main Agricultural Research Station, University of Agricultural Sciences,

Dharwad-580 005, Karnataka, India; 2ICRISAT, Kano, Nigeria

\*pujer\_851@rediffmail.com

Stem and pod rot caused by *Sclerotium rolfsii* is the major constraint to groundnut production in many groundnut growing regions of the world. Paucity of suitable field screening methods to identify sclerotium resistant genotypes hinders the progress of resistance breeding. A sick plot with high inoculum load of *Sclerotium rolfsii,* was established. A total of 165 sergeants derived from TAG 24 (adopted but susceptible variety) ⋅ R 9227 (stem rot resistant variety) were screened for resistance to stem and pod rot. The F5 and F6 generationswere grown in the sick plot to confirm their reaction to stem and pod rot. Among different parameters, variation was highly heritable for yield per plant, disease incidence parameters potential for selection under disease epidemics. Strong negative association between disease incidence and yield per plant revealed the importance of disease incidence in determination of yield per plant under epiphytotic conditions. The higher number of superior segregants observed for pod weight per plant (26), oil content (21), test weight (19) and shelling percentage (8) and disease at harvest (6) were compared to both the parents. None of the genotypes showed complete resistance. It is unlikely that highly resistant genotypes to neurotropic pathogen like *Sclerotium rolfsii* would be identified. However information obtained on genotypic variance, heritability, genetic advances and association of disease with yield and lines selected with considerable sclerotium resistance with good yield attributing characters (6) can be utilized in future breeding programs.

**Cloning and functional analysis of peanut *SAD* promoter**

Lei Shi\*, Fei-yan Qi, Suo-yi Han, Bing-yan Huang, Wen-zhao Dong, Feng-shou Tang, Xin-you Zhang

Industrial Crops Research Institute, Henan Academy of Agricultural Sciences / Key Laboratory of Oil Crops in Huanghuaihai Plains, Ministry of Agriculture / Henan Provincial Key Laboratory for Oil Crops Improvement, Zhengzhou 450002, China

\*leis100@163.com

The stearoyl-acyl carrier protein desaturase (SAD) plays key roles in determining the ratio? of saturated to unsaturated fatty acids in higher plants. The 720 bp sequence of promoter of peanut *SAD* was isolated from the genomic DNA of peanut cultivar Yuhua 9326 by nested PCR using genomic walking method. Using 5′ RACE (Rapid Amplification of cDNA End), the transcription start site was localized on -138 bp from the translation initiation codon ATG. Bioinformatics analysis indicated that *SAD* promoter contained some light responsive elements, hormone responsive elements and enhance-like elements as well as CAAT box and TATA box. To study the function of this promoter, the binary expression vector pBI121-SAD containing SAD-*GUS* fusion gene was constructed and introduced into Arabidopsis and transiently expressed in peanut, respectively, by *Agrobacterium*-mediated transformation method. Histochemical staining analysis of T2 transgenic *Arabidopsis* plants showed that the *GUS* gene mainly expressed in roots, leaves, stems and seeds, which are basically consistent with the expression in peanut. The only difference of GUS activity between *Arabidopsis* and peanut was that Histochemical staining observed in seed coat and mesophyll of peanut but not in *Arabidopsis*.

**Use of SNP technology for marker-assisted breeding using peanut interspecific introgression lines**

Mark D. Burow1,2\*, Ratan Chopra2, Roshan Kulkarni3, Theophilus Tengey3, Jennifer Chagoya4, Jeffrey Wilson5, Michael R. Baring5, A. Hillhouse6, Charles E. Simpson7

1Texas A&M Agrilife Research; 2Texas Tech University, Dept. of Plant and Soil Science; 3Texas Tech University, Dept. of Plant and Soil Science; 4Texas A&M Agrilife Research; 5Texas A&M Agrilife Research; 6Dept. of Molecular and Cellular Medicine; 7Texas A&M University, Texas A&M Agrilife Research, USA

\*[mburow@tamu.edu](mailto:mburow@tamu.edu)

Peanut wild species are a valuable source of alleles; however, linkage drag can reduce the utility of introgression lines.  Marker-assisted backcrossing  (MAB) offers considerable promise but has been limited by the slow pace of marker analysis using previous marker methods.  High-throughput SNP analysis offers an alternative, promising both rapid analysis as well as a large number of potential alleles.  We have developed two populations to date, with additional populations at earlier stages of development.  An A genome diploid population (*A. duranensis* 38901 x *A. cardenasii* 10017) has been advanced to the F4 generation, and has been mapped using transcriptome-based SNPs.  A total of 144 SNPs were mapped on the F2 generation on a Roche LightCycler 480 and/or Fluidigm Biomark HD, and QTLs were identified for growth habit and leaf morphology.  DNA has been extracted from the F3 generation, and work is underway on use of genotype-by-sequencing.  B and K-genome populations are also under development.  A BC3F1 introgression population had been mapped previously using RFLP markers and additional QTLs for nematode resistance were identified.  SSR markers recently have been used to identify QTLs for oil content and composition and leaf spot resistance.  We are currently working on developing a SNP-based map of this population, with the expectation that these markers can be used for development of near-isogenic introgression lines, and for QTL analysis of hybrids involving the BC3 materials.

**CRISPR/Cas9-mediated genome editing in peanut**

Mei Yuan1,4, Phat Dang2, Charles Chen3, C.S. Prakash4, Guohao He4\*

1Shandong Peanut Research Institute, Qingdao, China; 2USDA/ARS, Dawson GA 39842; 3Auburn University, Auburn AL 36830; 4Tuskegee University, Tuskegee AL 36088

\*hguohao@mytu.tuskegee.edu

The clustered regularly interspaced short palindromic repeats (CRISPR) – associated RNA-guided Cas9 nucleases (CRISPR/Cas9) system has recently provided a powerful tool for the targeted gene disruption to study gene function. The system has enabled to specifically break the targeted double-stranded DNA, from where resulting DNA repair to induce mutations by insertion/deletion of nucleotides (indels). Here, we describe the use of CRISPR/Cas9 in peanut genome and targeted specific nucleotide sequences of the fatty acid desaturase 2 (FAD2) for disruption. PCR amplification, cloning, and sequencing of PCR amplicons revealed indels in the targeted FAD2 genes. The results showed for the first time that CRISPR/Cas9 mediated gene editing is achievable in peanut, stimulating further functional genomics related studies in peanut.

**Redox systems are a potential link between drought stress susceptibility and the**

**exacerbation of aflatoxin contamination in crops**

Liming Yang1,2, Jake C. Fountain1,2, Xinzhi Ni3, Pingsheng Ji2, Brian T. Scully4, Robert C. Kemerait2, Robert D. Lee5, Baozhu Guo1\*

1USDA-ARS Crop Protection and Management Research Unit, Tifton, GA, USA; 2Department of Plant Pathology, University of Georgia, Tifton, GA, USA; 3USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA, USA; 4USDA-ARS U.S. Horticultural Research Laboratory, Ft. Pierce, FL, USA; 5Department of Crop and Soil Sciences, University of Georgia, Tifton, GA, USA

\*baozhu.guo@ars.usda.gov

Drought stress aggravates *Aspergillus flavus* infection and aflatoxin contamination in oilseed crops such as peanut and maize. Reactive oxygen species (ROS) are produced in plants in response to abiotic and biotic stresses as a means of defense. In the host plant-*A. flavus* interaction under drought conditions, the roles of ROS production remain unclear. In order to investigate the possible reasons of drought induced aflatoxin contamination, several maize lines with differential resistance to drought and aflatoxin contamination were subjected to drought treatment. Proteomic and enzymatic activity analyses demonstrated that drought tolerance was associated with different responsive patterns in redox homeostasis and ROS metabolism processes in developing kernels and seedling leaves. Drought stress triggered more rapid increases in the expression of ROS-scavenging enzymes such as superoxide dismutase, glutathione S-transferase, and antioxidant enzymes such as thioredoxin and peroxiredoxin in sensitive lines in comparison to tolerant lines. In addition, the potential roles of host derived-ROS in stimulating aflatoxin production were explored using a modified kernel screening assay. Kernels were with and without pre-incubation in high humidity for 3 days to induce differential ROS accumulation in kernel tissues followed by *A. flavus* inoculation. Pre-incubation resulted in reduced aflatoxin contamination with the drought tolerant line exhibiting greater aflatoxin resistance. Given that hydrogen peroxide (H2O2) have been shown to stimulate the production of aflatoxin in *A. flavus in vitro*, the above results imply that drought-induced ROS increases in crops may act as important inducers of aflatoxin production in *A. flavus*. Continuing research will further examine the role of these redox systems in peanut and corn explore the crosstalk signaling pathways between these crops and the fungus.

**Construction of a SNP-based genetic linkage map by ddRADseq and QTL detection for resistance to late leaf spot and plant type-related traits in peanut**

Xiaojing Zhou, Youlin Xia, Xiaoping Ren, Yulin Chen, Li Huang, Boshou Liao,

Yong Lei, Liyin Yan, Huifang Jiang\*

Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences

Wuhan, Hubei, 430062, China

\*zhouxiaojing@caas.cn

High density genetic maps are important in peanut (*Arachis hypogaea* L.) for providing precise information of the QTLs controlling traits of interest. In this study, using the ddRADseq data combined with bioinformatic analysis, we detected 53,257 SNPs between Zhonghua 5 and ICGV86699, of which 14,663 SNPs were also detected in their RIL individuals, and 1,765 of the obtained polymorphic markers met the requirements for use in construction of a genetic map. One linkage map was constructed, which was comprised of 1,685 marker loci, including 1,621 SNPs and 64 simple sequence repeat (SSR) markers. The map displayed a distribution of the markers into 20 linkage groups (LGs A01–A10 and B01–B10), spanning a distance of 1,446.7 cM. For the late leaf spot and plant type-related traits, a total of 67 QTLs with LOD >2.5 were detected in multi-environments. Each QTL explained 3.41–19.12 % of the phenotypic variance. Six consensus QTLs were detected in at least 2 environments. Nine stable major QTLs accounting for over 10% of phenotypic variation were included in six optimal clusters on the A5, A9 and B6 chromosomes. The first high density SNP-based linkage map for peanut can serve as a reference map for cultivated *Arachis* species and contribute to the assembly of a reference genome sequence for peanut. These putative QTL may be promising for further fine-mapping and genetic improvement through marker-assisted breeding.

**Deep sequencing-based comparative transcriptional profiles for response to aflatoxin production by *Aspergillus flavus* in resistant and susceptible peanut genotypes**

Houmiao Wang, Yong Lei, Liyun Wan, Liying Yan, Xiaoping Ren, Yuning Chen,

Xiaojing Zhou, Huifang Jiang, Boshou Liao\*

Oil Crops Research Institute of Chinese Academy of Agricultural Sciences

Wuhan, Hubei 430062, China

\*lboshou@hotmail.com

Peanut (*Arachis hypogaea* L) is an important source of vegetable oil and protein worldwide. Aflatoxin contamination seriously affects peanut industry development and human health. Though diverse reaction to aflatoxin production has been reported in peanut germplasm lines, the molecular mechanism of the resistance has not been well understood. In this study, RNA-seq was used for global transcriptome profiles of aflatoxin-resistant (Zhonghua 6) and susceptible (Zhonghua 12) peanut seeds under *Aspergillus flavus* infection. A total of 128.72 Gb of high-quality data were generated and assembled into 128,725 unigenes (average length 765 bp). About 62,352 unigenes (48.43%) were annotated in at least one of the Nr, Nt, Swiss-Prot, KO, Pfam, GO and KOG databases, and more than 93% of the unigenes were expressed in the samples. Among the obtained 30,143 differentially expressed unigenes (DEGs), 842 potential defense-related genes, including PGIPs, NBS-LRRs, LRR-RLKs, MAPKs, WRKYs, ERFs, bZIPs, PR proteins and crucial factors of other defense-related pathways were identified , those genes might be related with aflatoxin production in peanut. Notably, DEGs involved in phenylpropanoid-derived compounds biosynthetic pathway were induced to higher levels in the resistant genotype than the susceptible one. Furthermore, phenylpropanoid, flavonoid, and stilbenoid biosynthesis pathways were enriched only in the resistant Zhonghua 6. This study provided the first comprehensive insight into the transcriptome for response to aflatoxin productioninpost-harvest peanut seed.

**Integration of rapid phenotyping and genotyping tools for peanut genetic improvement**

D.J. O’Connor1\*, R.C.N. Rachaputi1, R.J. Henry1, A. Furtado1, G.C. Wright2

1Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, 4072; 2Peanut Company of Australia, Kingaroy, QLD, 4610

\*oconnor@uq.edu.au

Blanchability, propensity of the testa to be removed from the kernel following rapid heat treatment, is a key breeding trait for the cultivated peanut (*Arachis hypogaea).*  Blanchability is an ideal candidate for marker assisted selection (MAS) as it is difficult to phenotype, highly heritable, genotype specific and has a low genotype by environment interaction. Currently, due to the existing phenotyping technique, many undesirable lines are well progressed in a breeding program, only to be discarded after exhibiting poor blanchability at the F5 or F6 generation.  Progress of MAS in the cultivated peanut has been slow due to its large genome size, 2800Gbp, complex nature, it is an alleotetraploid, and  low genetic diversity in the domesticated species.  The reference genome for the cultivated peanut is still in development but annotated references have been released for the two diploid progenitors, *Arachis duranesis and Arachis ipaensis.*  99 lines from the US peanut minicore collection have been phenotyped for blanchability with significant variability identified, between 95% and 45%. A pooled DNA sample from a selection of excellent and poor blanching accessions has been developed. These two DNA pools have been enriched using probes developed from the annotated diploid reference, in collaboration with Roche Nimblegen. The enriched DNA has then been next generation sequenced using the Illumina platform in order to develop functional DNA markers for the trait.  It is expected this novel protocol will increase the efficiency of peanut breeding programs to select for other difficult to phenotype breeding traits.

**Evaluation of intensity and duration of seed dormancy in a recombinant inbred population derived from Spanish bunch genotypes**

Y.B. Naganagoudar1\*, P.V. Kenchangoudar1, B.N. Motagi1,2, M.V.C. Gowda1, H.L. Nadaf1, S. Pujer1

1Oilseeds Scheme, Main Agricultural Research Station, University of Agricultural Sciences,

Dharwad-580 005, Karnataka, India; 2ICRISAT, Kano, Nigeria

\*[yallurajgouda@gmail.com](mailto:yallurajgouda@gmail.com)

Pre-harvest sprouting in groundnut (*Arachis hypogaea* L.) seeds belonging to subspecies *fastigiata* (Spanish bunch)is undesirable. Since it leads to *in vitro* germination resulting in substantial loss of seeds, both in quantity and quality. A short period of dormancy is therefore desirable in the sub-species to reduce such losses. Evaluation of fresh seed dormancy was conducted for two seasons to determine the intensity and duration of dormancy in recombinant inbred population with 268 RILs  developed from crosses involving moderately dormant (GPBD-4) and non-dormant (TAG-24) parents. The intensity of dormancy ranged from 0 to 100% in summer season while 0 to 90% in *kharif* season.  There was large variation in the intensity of dormancy which could be related to genetic differences between the entries tested. RIL nos. 165, 259, 160, 172, 209, 254, 213, 247, and 248 recorded very high values (> 70 %) of intensity of dormancy in both the seasons. The variation for dormancy in terms of duration as revealed by germination of 70% (G 70 estimates) was subsequently large as compared to the intensity of dormancy among the RILs. The RIL nos. 5, 40, 84, 165, 183, 209, 213, 248, 254, 259 and 265 were found to have more than two weeks of dormancy (based on G70 count) in both the seasons. These dormant lines can be utilized in breeding for fresh seed dormancy under Spanish background.

**Response of groundnut mini core collection to iron deficiency chlorosis**

Omprakash Kumar Singh1, S.K. Pattanashetti2\*, B.D. Biradar1, G.K. Naidu1,

H.D. Upadhyaya2, M.K. Pandey2, R.K. Varshney2

1Department of Genetics and Plant Breeding, College of Agriculture, Vijayapur 586 101, U.A.S., Dharwad, India; 2International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, India

\*s.pattanashetti@cgiar.org

Groundnut utilizes strategy I mechanism (legumes) for iron uptake and utilization, and also found sensitive to iron deficiency. In India, more than one-third of the soils are calcareous and spread mostly in low rainfall areas of the western and central parts of the country, where groundnut is a major crop. Since calcareous soils are deficient in available iron, iron deficiency chlorosis (IDC) is more prevalent in Saurashtra region of Gujarat, Marathwada region of Maharashtra, and parts of Rajasthan, Tamil Nadu and Karnataka states in India causing considerable reduction in pod yield. Towards identifying IDC resistance sources in groundnut, a representative subset of geographical diversity i.e., mini core collection (184 accessions) was evaluated along with checks in a field experiment under iron-deficient calcareous soils at College of Agriculture, Vijayapur. They were assessed for IDC resistance associated traits like visual chlorotic rating (VCR) [1-5 scale: 1=No chlorosis, 5=Severe chlorosis] and SPAD chlorophyll meter reading (SCMR) at five different stages (20, 40, 60, 80, and 100 days). Severity of IDC was highest at 60 days evident from overall higher VCR scores and lesser SCMR values among all genotypes. In the mini core collection, five accessions (ICG #5051, 6667, 6766, 10890, 11651) were found as ‘resistant’ (VCR <2.0), while 80 as ‘moderately resistant’ (VCR >2 to 3.0). More number of resistant and moderately resistant accessions came from Virginia bunch/ runner types suggesting that subspecies *hypogaea* is the potential source for resistance to IDC. The identified sources need to be confirmed and utilized in breeding programme towards development of high yielding groundnut cultivars with IDC resistance.

**Phenotyping of a RILs population derived from a synthetic amphidiploid for peanut smut resistance**

F. de Blas1\*, S. Soave 2, L. Torres1, C. Oddino2, 3, M. Pepermans1, J. Soave2, M. Buteler 2

1F.C.A. – U.N.C.; 2Criadero El Carmen; 3F.A.V. – U. N. R. C.

\*fdeblas@agro.unc.edu.ar

Peanut smut (*Thecaphora frezii*) is causing major damages to the peanut crop in Argentina, particularly in Córdoba the main producing region. Criadero El Carmen has developed a 94 recombinant inbreed lines population derived by single seed descent from the cross of the synthetic amphidiploid JS.S\*-1806—[(*A. correntina* x *A. cardenasii*) x *A. batizocoi*]4x—and the high oleic elite peanut line JS.17304-7-B. The parent JS.S\*-1806 has been tested as immune to the smut while the *A. hypogea* parent is highly susceptible as all cultivars used in Argentina. The objective was phenotyping the RILs population for peanut smut resistance to study the inheritance of the trait for characterizing its genetic architecture and developing a system of molecular markers to assist in the introgression of resistance genes from wild *Arachis* species used. In 2014/2015 season, the 94 RILs and its parents were planted in a managed environment fivefold smut teliospore concentration usually find in any peanut crop field in the region. At the end of the season all pods were evaluated for the incidence of peanut smut—% of infected pods—and disease severity measured on a 0 to 4 scale. Statistical analysis was based on a mixed model with genotypes as fixed effects. The DGC test discriminated (p≤0,05) three genotypes groups: (A) comprising the susceptible parent, showing a high to medium disease incidence; (B) showing a medium incidence level; and (C) with a very low level—below 1% of incidence—or completely sound, equal to the immune amphidiploid.

**Screening of groundnut interspecific derivatives for resistance to *Sclerotium rolfsii***

M. Balaraju1, P.V. Kenchangoudar1\*, B.N. Motagi1, S.S. Adiver1, M.V.C. Gowda1,S. Pujer 1

1Oilseeds Scheme, Main Agricultural Research Station, University of Agricultural Sciences,

Dharwad-580 005, Karnataka, India

2ICRISAT, Kano, Nigeria

\*pvkgoudar@gmail.com

*Sclerotium rolfsii* is a major constraint for production in most of the groundnut growing areas in India inflicting 28-30 per cent yield losses annually. Irrigated groundnut grown in the post-rainy and summer seasons in India is often infected by this pathogen. The 54 interspecific derivatives and 6 recombinant inbred lines (TAG-24 x R-9227 cross) along with 6 check varieties (Dh-86, Dh-216, ICGV 91114, Dh-3-30, R-9227 and TAG-24) were screened for *S. rolfsii* resistance in sick plots during summer and *Kharif* 2012 seasons. In summer 28 lines and 32 lines in *Kharif* showed highly resistant reaction to stem rot disease. High genetic variability and heritability was observed for disease and yield attributes viz., number of pods per plant, pod yield, harvest index, disease incidence at harvest, disease severity and disease spread irrespective of seasons. Per cent disease incidence at harvest was having strong significant negative association with dry pod yield per plant in both seasons. The interspecific derivatives viz., ICGV 3649-1, ICGV 4368-1, ICGV 3727-4, ICGV 34-1 and  ICGV 4670-7 and  recombinant inbred lines viz., RIL 3-14, RIL 6-1 and RIL 6-28 had desirable combination of high level of stem rot resistance and good agronomic attributes. These promising lines can been tested in trials over locations to confirm their superiority and utilized in breeding for Sclerotium disease resistance.

**Molecular cloning and characterization of phospholipase D from peanut (*Arachis hypogaea*)**

Silong Chen1\*, Boshou Liao2, Yurong Li1

1Hebei Provincial Laboratory of Crop Genetics and Breeding, Cereal and Oil Crop Institute, Hebei Academy of Agricultural and Forestry Science, Shijiazhuang, 050031, China. 2Key Laboratory of Biology and the Genetic Improvement of Oil Crops, Ministry of Agriculture, Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan, 430062, China.

\*chsl99@163.com

Phospholipase D (PLD) is a kind of important enzymes in signal transduction of biological membrane and plays a crucial role in responding to drought stress in plant. Some evidences demonstrated the specificity of PLDs in signal transduction with plant species and cellular processes. In our previous studies, a novel *PLD* gene, *AhPLDα3*, was isolated from peanut (*Arachis hypogaea*) via cDNA library screening. The full-length cDNA and genomic DNA sequence of *AhPLDα3* were cloned; our data demonstrated that *AhPLDα3* cDNA was 2717 bp in length with a complete open reading frame of 2439 bp which encoded a polypeptide of 812 amino acids with a predicted molecular mass of 93.1 kD and a theoretical isoelectric point (*pI*) of 6.42, and its genomic sequence was 3031 bp. *AhPLDα3* was composed of three exons and two introns with typical GT-AG sequence at the splice sites. The two highly conserved catalytic HXKXXXXD (HKD) motifs, which are key amino acid residues related to the PLD activity, are encoded by two highly conserved exons. Phylogenetic analysis indicated *AhPLDα3* showed a low similarity to other PLDαs from plants, such as *Arabidopsis thaliana*, *Ricinus communis*, *Jatropha curcas* and *Glycine max*. The gene expression of *AhPLDα3* was strongly stimulated by water deficit. More importantly, *AhPLDα3* presented a remarkable stability of expression in conditions of progressive drought stress in peanut. Therefore, *AhPLDα3* could be greatly important for peanut to respond to drought stress. Additionally, *AhPLDα3* from peanut will provide an additional candidate gene for drought-tolerant crops improvement.

**Chromosome structural stability but canalized amphiplasty in**

**AABB allotetraploids of *Arachis***

G. Seijo1,2\*, L. Chalup1, S. Samoluk1, A.P. Fávero3, G. Robledo1,2

1Instituto de Botánica del Nordeste; 2Facultad de Ciencias Exactas y Naturales, Universidad Nacional del Nordeste, Corrientes; 3Embrapa Pecuária Sudeste, São Carlos-SP, Brazil.

\*seijo@agr.unne.edu.ar

Allopolyploidy is an important evolutionary mechanism in plants. Numerous genetic and epigenetic mechanisms have been shown to generate the wide range of structural and functional genome modifications associated with merged genomes arising from polyploidy events. However, to date little is known about the genome interactions that occurred in the AABB tetraploids of *Arachis*. In this report, we cytologically analyzed three AABB polyploids: the cultivated peanut, the wild *A. monticola* [both with spontaneous origin (*A. duranensis* x *A. ipäensis*) 4x]and one reciprocal synthetic amphidiploid (*A. ipäensis* x *A. duranensis*)4x. The patterns (number and position) of heterochromatic DAPI+ bands and the 45S and 5S rDNA mapped by FISH were highly conserved among the polyploids and exactly reproduced the sum of those observed in the diploid progenitors. Telomeric probes hybridized always at the end of the chromosomes and did not reveal any structural rearrangement. Moreover, the occurrence of intergenomic translocation was not evidenced by GISH analysis. However, in the three polyploids analyzed the activation of the NORs was biased in favor of those belonging to the A genome (*A. duranensis*). Our results showed a clear pattern of additivity with a strong structural quiescence at the chromosome level. However, the pattern of rDNA activation demonstrates that rapid functional changes occurred in the AABB allopolyploids, which involve the differential inactivation of the B genome nucleolar organizing regions (NOR). Moreover, the differential inactivation of the B genome NORs occurred irrespective of the species that acted as maternal donor, suggesting a canalized epigenetic control of these loci.

**Genetic mapping of microsatellite markers based on genome survey sequences**

**and expressed sequence tags in *Arachis* species**

Li Huang, Bei Wu, Jiaojiao Zhao, Xiaoping Ren, Yuning Chen, Xiaojing Zhou,

Boshou Liao, Huifang Jiang\*

Oil Crops Research Institute (OCRI) of Chinese Academy of Agricultural

Sciences (CAAS), Wuhan, Hubei, 430062, China

\*[peanutlab@oilcrops.cn](mailto:peanutlab@oilcrops.cn)

Microsatellite or simple sequence repeat (SSR) is one of the most widely distributed polymorphisms that has been largely utilized to assess genetic diversity and genetic mapping of important traits in plants. However, investigation of microsatellite characteristics and available number of high-quality SSR markers remained limited in *Arachis* species. In the present study, 65,111 genome survey sequences (GSSs) and 281,115 expressed sequence tags (ESTs) were downloaded from GenBank database and 7 *Arachis* species were involved. Among these *Arachis* species, 9.83% to 42.47% of the sequences contained microsatellite sequences, where the dinucleotide and trinucleotide repeat motifs were the most abundant repeat type. The microsatellite characteristics of these *Arachis* species were highly similar, which suggests that the microsatellite distribution across different *Arachis* species is  evolutionarily conservative. A total of 2,590 new SSR markers were identified based on publicly available GSSs and ESTs. The genetic polymorphism was tested for each SSR and no significant correlations of which were found with SSR motifs, repeat number or repeat length. With a subset of high-quality 540 new SSRs as well as 105 public anchor markers, a genetic linkage map of an *A. hypogaea* RIL population was constructed spanning 1711.47 cM in total length and 2.65 cM between flanking adjacent SSR markers. The SSR-based genetic map would give new insights of the genetic determinants of important agronomic traits and facilitate marker-assisted breeding in peanut.

**Combining biotech and conventional methods to develop high-oleic,**

**Sclerotina blight resistant peanut cultivars**

Kelly Chamberlin1\*, Ning Wang3, Rebecca Bennett1, John Damicone2

1USDA ARS, Stillwater, OK; 2Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK; 3Department of Biosystems and Agricultural Engineering, Oklahoma State University, Stillwater, OK

[\*kelly.chamberlin@ars.usda.gov](mailto:*kelly.chamberlin@ars.usda.gov)

Cultivated peanut is the second most economically important legume crop throughout the United States and the third most important oilseed in the world.  Each peanut growing region has a unique set of economical, environmental and biological challenges that threaten sustainable production.  In the Southwestern United States, peanut breeding programs focus on developing peanut cultivars that are resistant to *Sclerotinia minor* Jagger (*S. minor*), the causal agent of Sclerotinia blight and a major threat to peanut production.   Peanut manufacturers, shellers and consumers are increasingly insistent on high-oleic peanuts and peanut products due to their extended shelf-life and improved health benefits.  In fact, in the Southwestern U.S., contracts are only offered to those growers producing a high-oleic variety.   We have developed a breeding strategy which incorporates biotech and conventional methods, enabling targeted screening high-oleic content and Sclerotinia blight resistance for desired traits and improved efficiency when developing high-oleic, Sclerotinia blight resistant peanut cultivars.  Here we discuss that strategy, the methods used, and cite examples of successful implementation resulting in cultivar release.

**Development of novel SSR makers within resistance gene analogues**

**for groundnut (*Arachis hypogaea* L.)**

Y. Amaravathi\*, R.P. Vasanthi, K. Raja Reddy

Acharya N.G. Ranga Agricultural University,

Regional Agricultural Research Station, Tirupati-517502

[\*dryellaridreddy@gmail.com](mailto:*dryellaridreddy@gmail.com)

Cultivated groundnut or peanut (*Arachis hypogaea* L.) is an amphidiploid leguminous annual crop proposed to be derived from natural hybridization of two wild progenitors *A. duranensis (*AA*)* and *A. ipaënsis* (BB) followed by chromosome doubling with a genome of 2891 Mbp. Peanut yield and quality are severely constrained by a wide variety of fungal, bacterial, viral, and nematode pathogens. With the aim to increase the number of functional markers in resource poor crop like cultivated groundnut, resistance gene analogues (RGAs) in the public database were utilized for the development of novel RGA derived simple sequence repeat (SSR) markers. These RGA derived SSRs would be more useful in targeted marker assisted breeding for biotic stresses as these are already linked to disease resistance traits. In this context, a total of 861 RGAs reported in different crops including groundnut were analyzed for SSRs with SSRIT (Simple Sequence Repeat Identification Tool) with maximum length of repeat as decamer and minimum number of repeats as five were selected. Analysis with SSRIT resulted in identification of 38 (4.41%) RGAs containing 42 SSR motifs, i.e., four of them each containing two SSRs. A greater number of SSRs were derived from RGAs of Arachis genome in comparison with RGAs of other crops. Among different SSR motif-classes, two thirds were found to be tri-nucleotide repeats (66.67%) followed by di-nucleotide repeats (28.57%). The dinucleotide GA/CT (11.9%) and TCT/AGA (9.5%) were the most abundant repeat-motifs. The primers were designed for SSRs using Primer 3 software. These markers were validated with e-PCR. These RGA-SSRs would facilitate the targeted research in marker-trait association for various biotic stresses in groundnut.

**Genotype and environment influence on antioxidant expression and**

**antioxidant related proteins in *Arachis hypogaea***

Yan Yee Poon1\*, Sridevi Muralidharan1, Graeme Wright2, Paul Haynes3, Alice Lee1

1ARC Training Centre for Advanced Technologies in Food Manufacture, School of Chemical Engineering, University of New South Wales, Kensington, NSW 2052, Australia; 2Peanut Company of Australia, Kingaroy, QLD 4610, Australia, 3ARC ITTC for Molecular Technology in the Food Industry, Macquarie University, Sydney, Australia

[\*yannie.poon@student.unsw.edu.au](mailto:*yannie.poon@student.unsw.edu.au)

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Consumers have been increasingly aware of human nutrition for the past few decades. Recent trends have been focused towards functional traits of foods. Peanuts contain antioxidants, which protect against oxidative stresses commonly present in inflammation, cellular respiration, cancers neurodegenerative disorders and cardiovascular diseases. The most definitive method for breeding stable lines of higher antioxidant producing peanuts cultivars is through the inclusion of high antioxidant and related genetic material. With the use of recombinant inbred lines (RILs) developed from the hybridization of breeding line (D147-p3-115) and cultivar (Farnsfield), offspring with significant variability for total antioxidant expression were obtained. Select RILs (RIL parents, p27-272, p27-036, p27-362) with very high or very low antioxidant expression were chosen for quantitative proteomics analysis in aims of discovering high and low antioxidant expression related proteins. Varying expression between the high and low antioxidant cultivars were identified in relation to specific biological pathways and metabolic changes in RILs were established. Changes in expression levels of enzymes such as chalcone synthase, catalase and 7s globulin were found and are of specific interest being stilbene synthesis, antioxidant and allergen related proteins. The identification of these proteins will assist in the detection of possible antioxidant biomarkers and in turn allow breeders select for more desirable cultivars for the breeding of high antioxidant lines.

**Host range of the peanut root rot pathogen *Fusarium neocosmosporiellum***

Kyle Wenham

School of Agriculture and Food Science, The University of Queensland, Gatton, Queensland, 4343, Australia

\*k.wenham1@uq.edu.au

*Fusarium neocosmosporiellum* is the causal pathogen of the emerging soilborne disease Neocosmospora root rot of peanuts (*Arachis hypogea*) and can be characterised by severe root and crown rot of peanut crops in Australia. *Fusarium neocosmosporiellum* has been reported as a pathogen of other leguminous crops such as chickpeas (*Cicer arietinum*) and soybeans (*Glycine max*). Twenty three cultivars of eight species of plants commonly included in broad-acre crop rotation systems with peanuts were inoculated by placing a mycelial plug of *F. neocosmosporiellum* against wounded and non-wounded stems below the cotyledonary node. All plants were maintained under glasshouse conditions at 21 - 30°C ± 2°C. Lesions developed around the site of inoculation for both the wounded and non-wounded plants for chickpeas, soybeans, peanuts, mung beans, cotton and wheat. Discolouration of the vascular tissue was observed for all of these species plus sorghum and maize. None of the plants exhibited typical field symptoms of the disease such as chlorosis and wilting and no root system damage or discolouration was apparent. *F. neocosmosporiellum* was reisolated from vascular tissue 10mm from the internal stem lesion of each plant species and isolated from the taproot of peanuts only.  While there was some progression of the disease in the infected plants, infection did not appear to influence plant growth and development therefore indicating *F. neocosmosporiellum* is able to successfully colonize non-susceptible host plants in an endophytic capacity and may contribute to the survival of the pathogen in cropping soils.

**Author Index**

**Name Page**

Abernathy, B. 1, 3, 5

Adiver, S.S. 24

Adnan, A.A. 17

Ado, S.G. 9

Agarwal, G. 3, 3

Ajeigbe, H.A. 17

Amaravathi, Y. 26

Arias, R. S. 6

Arun, S. 8

Asha, B. 17

Ayers, J.L. 7

Bajaj, P. 9

Balaraju, M. 24

Ballen-Taborda, C. 16

Barbour, J.A. 10

Baring, M.R. 19

Belamkar, V. 7

Bennett, R. 26

Bera, S.K. 7

Bertioli, D.J. 1, 1, 3, 16

Bhat, R.S. 17, 18

Biradar, B.D. 23

Bowen, K. 8

Brenneman, T. 5

Bryan, J. 10

Buckley, J.D. 10

Burow, M. 5, 7, 18

Buteler, M. 23

Butts, C. 5

Cannon, E. 4

Cannon, S. 2, 4

Chagoya, J. 7

Chagoya, J. 19

Chalup, L. 25

Chamberlin, K. 26

Chandrayudu, E. 14, 14

Chauhan, Y. 13

Chavarro, C. 3, 5, 5

Chen, C. 5, 20

Chen, C.Y. 8

Chen, Y. 4, 6, 21, 21, 25

Chitikineni, A. 9

Chopra, R. 7, 19

Chopra, R. 19

**Name Page**

Chu, Y. 2, 5, 16

Clevenger, J. 2, 3, 3, 5

Coates, A.M. 10

Cruickshank, A. 12

Culbreath, A. 5

Damicone, J. 26

Dand, P.M. 8

Dang, P. 20

Danquah, E. 9

Dash, S. 4

de Blas, F. 23

Desmae, H. M. 17

Devi, R.S.J. 15

Dickerson, J. 4

Dilly, J.L. 11

Dobariya, K. 7

Dong, W. 11, 19

Duke, M. 2

Echekwu, C.A. 9, 17

Farmer, A. 4

Faustinellia, P. 6

Fávero, A.P. 25

Fengshou, T. 11

Fleischfresser, D. 12

Fountain, J.C. 9, 20

Furtado, A. 22

Gao, D. 1

Gowda, M.V.C. 17, 18, 18, 22, 24

Guimaraes, L. 2

Guo, B. 5, 9, 20

Hagan, A. 8

Han, S. 19

Haynes, P. 27

He, G. 8, 20

Henry, R.J. 22

Hillhouse, A. 19

Holbrook, C. 5, 7, 16

Howe, P. 10

Huang, B. 11, 19

Huang, L. 4, 6, 21, 25

Huang, W. 2, 4

Ishiyaku, M.F. 9

Isleib, T. 5

Jackson, S.A. 1, 1, 3, 3, 5, 5, 16

**Name Page**

Janila, P. 3, 7, 17

Jayale, A. 9

Ji, P. 20

Jiang, H. 4, 6, 21, 21, 25

Kalberer, S. 4

Kemerait, R. C. 9, 20

Kenchangoudar, P.V. 18, 18, 22, 24

Khedikar, Y. 17

Kolekar, R.M. 17

Kottapalli, K. 8

Kottapalli, P. 8

Krosch, S. 13

Kulkarni, R. 19

Kumar, V. 9

Lamb, M. 5

Leal-Bertioli, S. 1, 1, 16

Lee, A. 27

Lee, D. 9

Lee, R.D. 20

Lei, Y. 4, 6, 21, 21

Li, B. 4

Li, Y. 24

Liang, X. 3

Liao, B. 4, 6, 21, 21, 24, 25

Livingston, D. 16

Maia, T. 2

Manivannan, N. 7

Manohar, S. 7

Mata, L. R. 11

M'biBertin, B. 7

Miao, L. 11

Mohammed, S.G. 17

Monyo, E. 17

Moretzsohn, M. 11

Morrissey, J. 16

Motagi, B.N. 17, 17, 18, 18, 22, 24

Muralidharan, S. 27

Nadaf, H.L. 7, 18, 22

Nagangoudar, Y.B. 22

Naidu, G.K. 18, 23

Naik, B.S.K 14, 14

Naik, K.S.S. 14, 14

Nayak, S. 9

Ni, X. 20

O'Connor, D.J. 12, 22

**Name Page**

Oddino, C. 23

Offei, S. 9

Ofori, D.E. 9

Okori, P. 17

Omoigui, L.O. 17

Owens, L. 12

Ozias-Akins, P. 1, 2, 3, 3, 5, 5, 16

Pandey, M.K. 3, 3, 7, 9, 23

Pappachan, A. 15

Pattanashetti, S.K. 23

Payton, P. 8

Pepermans, M. 23

Poon, Y. 27

Prakash, C.S. 20

Prathyusha, C. 14, 14

Premalatha, N. 7

Pujer, S. 18, 22, 24

Qi, F. 11, 19

Rachaput, R.C.N. 13, 22

Radhakrishnan, T. 7

Rajesh, A. P. 13, 14

Rao Gangadhara, V.S.G. 13

Rathore, A. 3

Reddy, K.R. 14, 14

Ren, L. 4

Ren, X. 4, 6, 21, 21, 25

Robledo, G. 25

Rowland, D. 8

Samoluk, S. 25

Sankara, P. 7

Sarvamangala, C. 17

Scheffler, B. 2

Schmitz, R. 1

Scully, B. T. 9, 20

Seijo, G. 25

Selvaraj, M.G. 7

Shasidhar, Y. 7

Shekoofa, A. 5

Shi, L. 11, 19

Shirasawa, K. 1, 17

Signh, O.K. 23

Simpson, C.E. 19

Sinclair, T. 5

Soave, J. 23

Soave, S. 23

**Name Page**

Sobolev, V. 6

Sonyal, S. 15

Stalker, H.T. 16

Sujay, V. 17

Sukruth, M. 17

Sunnam, R. 13

Tabo, R. 17

Tang, F. 19

Tang, Y.Y. 8

Tengey, T. 19

Tillman, B.L. 5, 15

Torres, L. 23

Umale, P. 4

Upadhyaya, H.D. 3, 17, 23

Usman, A. 9

Valls, J. F. M. 11

Variath, M. 7

Varshney, R.K. 3, 3, 2, 4, 7, 9, 17, 17, 23

Vasanthi, R.P. 7, 26

**Name Page**

Vemana, K. 14, 14

Vishwakarma, M. 7

Wan, L. 4, 6, 21

Wang, H. 6, 21

Wang, N. 26

Weeks, N. 4

Wenham, K. 27

Wilson, J. 19

Wright, G.C. 12, 13, 27

Wu, B. 25

Wu, Y. 4

Xia, Y. 21

Yan, L. 4, 6, 21, 21

Yang, L. 9, 20

Yuan, M. 20

Zhang, X. 11, 19

Zhang, X. 19

Zhao, J. 25

Zhou, X. 4, 6, 21, 21, 25