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Project Title: Introgression of pest and disease resistance genes from wild species into cultivated peanut lines and varieties.

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Layman`s summary
Root-knot nematodes (RKN) and late leaf spot (LLS) are very damaging to peanuts. Chemical treatments are expensive and hazardous; the best solution is the use of resistant varieties. Currently only a single source of resistance to RKN is available and there are serious concerns that this resistance could be broken. All widely grown US varieties are highly susceptible to LLS. We are also investigate new sources of strong pest and disease resistance, derived from wild species. We have generated new hybrids and induced chromosome doubling to create peanut-compatible hybrid allotetraploids, and investigated the genetics of resistance in a hybrid allotetraploid produced previously. These hybrids harbor resistance to LLS, RKN and various other pests that damage peanut production in the SE of the USA. We have located resistance genes on chromosomes, and developed DNA markers to some key regions of the peanut genome. Introgression of these resistances into peanut by backcross has started. The breeding lines produced will be a valuable resource for the development of new peanut cultivars with very strong pest and disease resistances.

Report of activities 2014 - 2016

i) Production of hybrids and allotetraploids

The objective of this activity was to obtain induced allotetraploids from wild species that harbor resistances to pests and diseases that affect the peanut production in the SE of the United States (Table 1). Over 1,500 pollinations were performed using six wild species in 2015 and over 1,000 in 2016.
Hybridity was confirmed for the following combinations using the technique of pollen staining:

A. valida PI 468154 x *A. stenosperma* V10309 (ValSten)
A. magna K30097 x *A. duranensis* V14167 (MagDur)
A. magna PI468337 x *A. stenosperma* V10309 (MagSten)

ValSten and MagDur hybrids were have been treated with 0.2% colchicine to induce tetraploidization. To date, seven pegs were identified. Seeds will be harvested in the beginning of winter and germinated in the spring. Plants from the hybrid MagSten have slow development, therefore, colchicine treatment will happen in the spring of 2017.

In 2016, over 1,000 crosses were done with the accessions:

A. gregoryi V6389 x *A. diogoi* 10602
A. magna K30097 x *A. diogoi* 10602
A. magna 30097 x *A. stenosperma* V10309
A. gregoryi V6389 x *A. villosa* V12812

Hybridity will be confirmed in the spring.

Table 1 – Disease and insect resistances reported for selected accessions (see Stalker et al., 2013 for references of evaluation work)

Species/diseases	*Aspergillus* (Aflatoxin)	*Cylindrocladium parasiticum*	Early leaf spot (*C. arachidicola*)	Late leaf spot (*C. personatum*)	Rust (*Puccinia arachidis*)	Groundnut rosette virus	Peanut Bud Necrosis Virus	Peanut Mottle Virus (PMV)	Peanut rust (*Puccinia arachidis*)	Peanut Stripe Virus (PStV)	Peanut stunt virus	Tomato-Spotted Wilt Virus (TSVW)	Peanut Root-Knot Nematode (*Meloidogyne arenaria*)	Northern Root-Knot Nematode (*Meloidogyne hapla*)	Armyworm (*Spodoptera* spp.),	Corn Earworm (*Helicoverpa armigera*)	Leafminer (*Aproaerema modicella*)	Leafhoppers (*Empoasca fabae*)	Groundnut aphid (*Aphis craccivora*)	Thrips
A genome																				
A. stenosperma			x	x	x	x			x			x	x	x		x	x	x	x	x
A. duranensis	x			x							x		x		x	x	x	x	x	x
A. correntina		x		x				x	x			x			x	x			x	x
A. diogoi			x			x	x		x	x		x	x	x	x	x		x	x	x
B genome																				
A. gregoryi				x	x															
A. magna			x	x	x															
A. valida		x	x	x					x											
K genome																				
A. batizocoi			x	x					x			x	x			x		x	x	x

A previously obtained diploid AB hybrid (*A. ipaensis* x *A. duranensis*) was treated with colchicine to induce tetraploidy. Three different tetraploidization events were obtained (Figure 1). These new allotetraploids have the same original parents as and are compatible with peanut. This opened new avenues for studies on gene expression, genetic and epigenetic.

Gene expression analyses were conducted to analyze genomic changes after hybridization and polyploidization. The new diploid hybrid, the newly induced allotetraploid and the natural allotetraploid peanut were evaluated, and compared with the diploid parentals *A. duranensis* and *A. ipaensis*. Analyses are underway. Preliminary results show that significant changes in gene expression happen after genome doubling.

Figure 1: New allotetraploid (*A. ipaensis* K30076 x *A. duranensis* V14167)^{4x}.

ii) Introgression of resistance to LLS and RKN into peanut

The previously obtained induced allotetraploid (*A. batizocoi* PI298689 x *A. stenosperma* PI666100)^{4x} harbors resistance to, among other pests, root-knot nematode (RKN, *Meloidogyne arenaria* Neal) and late leaf spot (LLS, *Cercosporidum personatum* Berk.). This allotetrapoid was crossed with peanut different peanut cultivars. An F_2 population with 250 individuals was generated and this population is being hosted at both UGA/Tifton and Athens.

The individuals were evaluated for RKN and LLS in the spring and fall 2014, respectively. For LLS, the method of detached leaf was used, allowing components of resistance to be evaluated on a quantitative basis (Figure 2). These experiments will be repeated in the fall 2015 and fall 2016. The evaluation for RKN was done three times. Greenhouse-based RNK bioassays will be performed using *M. arenaria* race 1 multiplied on susceptible tomato plants. 10-12 week old peanut plants will be inoculated with 5,000 - 10,000 eggs. Ten weeks after inoculation, eggs will be extracted from roots using 0·5% NaOCl (Hussey and Barker, 1973), stained with acid fuchsin and counted using a Peters slide under the microscope. Susceptibility will be measured by the gall index (1-5), number of eggs/ gram of root and nematode reproductive factor (Oostenbrink 1966).

Four selected F2 hybrids of *A. hypogaea* x (*A. batizocoi* x *A. stenosperma*)^{4x} are being be backcrossed with peanut cultivars.

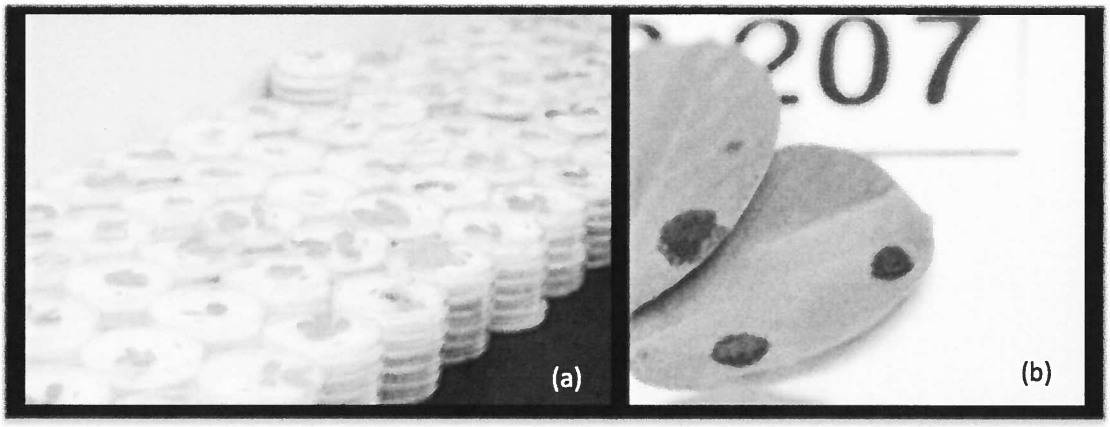

Figure 2. Bioassay of an F2 population derived from A. hypogaea cv Runner x (*A. batizocoi* x *A stenosperma*)^{4x}. a. Overview of inoculated leaves incubated in petri dishes. b. close up of a leaf with LLS symptoms.

iii) Development and use of molecular markers

With the aim of tracking wild species chromosome introgressions on the A and B genomes of cultivated peanut, markers were developed from two initial sets of 400 single nucleotide polymorphisms. This work used the newly generated peanut genome sequences of *A. duranensis* and *A. ipaensis*, that were used as proxies for the A and B genomes of peanut, respectively. SNPs were identified between *A.stenosperma* and *A. duranensis* (to tag introgressions on the A genome) and between *A. batizocoi* and *A. ipaensis* (to tag introgressions on the B genome). Markers were developed for the genotyping system Fluidigm®. The assays were outsourced to the research group in MARS Inc. Miami (Figure 3).

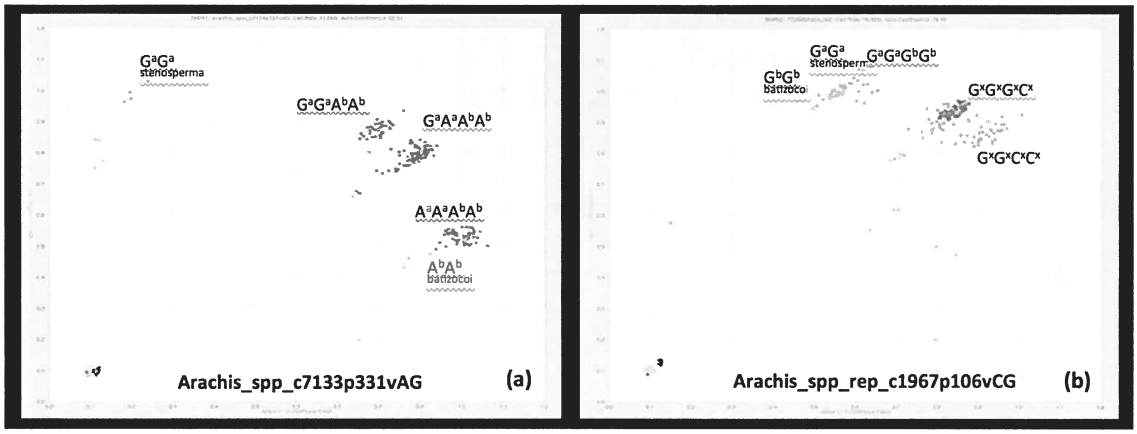

Figure 3. Examples of Fluidigm assays on the F_2 population *A. hypogaea* x (*A. batizocoi* x *A. stenosperma*)^{4x}. (a) Arachis_spp_c7133p331vAG, (b) Arachis_spp_rep_c1967p106vCG. Both markers were designed for the A-genome. Both generate five clusters, and identify the introgressed segments of *A. stenosperma* in the individuals tested.

iv) Generation of a genetic map

A total of 380 markers were genotyped on the A and B genomes of 96 F2 individuals of *A. hypogaea* x (*A. batizocoi* x *A. stenosperma*)^{4x}. This part of the work was funded by the Peanut Foundation. Genetic markers previously described were ordered into a framework genetic map. Ordering was aided by the sequence of peanut donors *A. duranensis* and *A. ipaensis* (Bertioli et al., 2016). For QTL analysis, phenotyping data of resistance to RKN was used. Traits evaluated in different trials or years were analyzed separately. QTLs were mapped using the composite interval mapping (CIM) method, proposed by Zeng (1994), using R-QTL. For the regions identified as linked to RKN resistance, easy-to-use, foreground KASP markers will be developed as described in Leal-Bertioli et al. (2016) for breeding foreground selection.

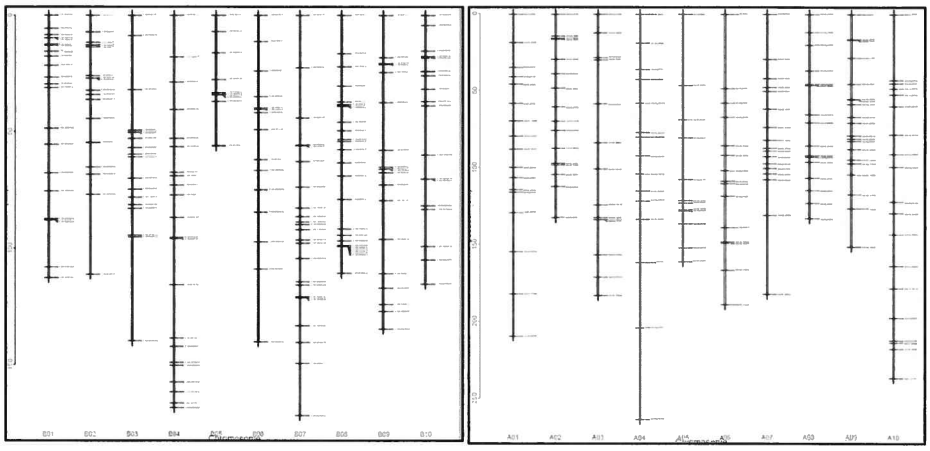

Figure 4: Genetic map of Runner x (*A. batizocoi* PI298689 x *A. stenosperma* PI666100)^{4x}.

v) Future directions:
Crosses will be continued. New hybrids will be confirmed in the spring 2016. Confirmed hybrids will be tetraploidized to produce peanut-compatible plants.

The lines developed from this program will be utilized in the cultivar development program to produce high yielding cultivars with high levels of resistance to both LLS and RKN. Lines and/or cultivars released will be registered in the American Journal of Plant Registrations. In addition, lines will be deposited in the USDA National Plant Germplasm System (NPGS) so that breeding programs and research scientists in other areas of the U.S. will have complete access. All information on markers associated with resistances will be made available in the website peanutbase.org, communicated in scientific meetings, such as APRES (American Peanut Research and Education Society), PAG (Plant and Animal Genetics), and published in scientific journals.

The materials produced as part of this project were used to leverage funding from the Peanut Foundation and Mars corporation for the reported molecular work which enhanced the utility of these materials.