Final Report for 2013 National Peanut Board funding to the Texas Peanut Producers Board.

I. Subject area: Breeding, Genetics and Molecular Genetics

a. Project Title: Molecular and Conventional Breeding to Increase Peanut Yields and Production Efficiency by Developing Breeding Lines with Improved Drought and Heat Tolerance combined with Multiple Disease Resistance

b. Funding Year: 2013

c. Co-PIs:

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e. Funds Requested: $59,500.00

f. Locations: Lubbock, College Station, Stephenville, Yoakum, and Pearsall, Texas

g. i. Continuing Project:

II. Layman's Summary:

The Texas A&M AgriLife Research peanut breeding program continued work on combining early maturity with multiple disease resistance, transferring drought and salt tolerance into elite breeding lines, and finding DNA markers associated with any or all of these traits during the 2013 growing season.

Research on combining early maturity and multiple disease resistance was carried out by testing 50 advanced breeding lines in two replicated runner trials at two locations in West Texas and one location in South Texas. Over one half of the early-maturing breeding lines had yields equal to the released cultivars with 10 lines having numerically higher yields. Most promising was the fact that two of these lines yielded over 1,000 lbs/a better than Tamrun OL12 indicating improvement upon the yield of our next generation, early maturing breeding lines. Additionally, we tested another 57 early generation breeding lines developed from crosses between our best early maturing lines and Tamrun OL11 which has resistance to Sclerotinia blight and excellent grade characteristics. These lines went through two years of selection under heavy Sclerotinia blight pressure at Stephenville and this was the first year of yield testing on these materials. They were only tested at one location in West Texas due to limited seed availability. Several of the breeding lines yielded numerically higher than the either of the parents and one of the highest yielding lines tested about 85% mature compared to 54% for Tamrun OL11.

Completion of yield data on the U.S. peanut minicore collection screen demonstrated that several lines repeatedly outyielded checks by 20 to 25% under drought stress. Some of these are now being used as parents to combine drought tolerance with traits from current or soon to be released varieties. Association mapping using DNA markers and the minicore drought data identified that markers that were previously found to be associated with good field response were also associated with improved yield. These will be used for marker-assisted breeding for drought tolerance. We are growing out runner populations derived from minicore selections and current varieties or advanced breeding lines, and will begin marker-assisted selection for improved yield under drought. Evaluation of a different runner cross made for drought tolerance identified several breeding lines that significantly outyielded the checks under drought stress.

A sophisticated marker analysis of a wild species introgression population demonstrated that nematode resistance is controlled by eight genes, far more than the one known of before. This will allow for new breeding strategies to develop nematode-resistant varieties that will have greater stability of resistance to nematodes in the long run.

III. Project Summary:

We tested 50 advanced lines in 2013, which were developed for early maturity at 3 locations across the state. Many of the lines performed in the same statistical grouping as did the check cultivars, but five of the breeding lines had numerically higher yields (**Table 1**).

Table 1. Top Performing Entries from a Combined Analysis of Early Maturing Runner-type Breeding Lines in 2013.

Entry	Lbs. Pods/A	%TSMK
TXL080224-02	6575a	72.1d-h
TXL080251-04	6555ab	69.8k
TXL080244-03	6544ab	71.7e-j
TXL080243-06	6510ab	73.4b-e
TXL080256-02	6412a-c	73.0c-f

Tamrun OL07	6238a-d	73.8b-d
Flavor Runner 458	6124a-e	74.9b
Tamrun OL12	5511c-h	71.7e-j
Mean	5784	72.2
CV	14.7	2.37
p-value (0.05 level)	≤ 0.0001	≤ 0.0001

Our first generation early maturity release was Tamrun OL12 and it matured approximately two weeks earlier than conventional runner cultivars, and yields were comparable. We consider these breeding lines to be our improved second generation materials and, as shown in Table 1, several of these lines have the potential to yield over 1,000 lbs/a better than the original Tamrun OL12 release while maintaining its earliness. We will reduce the number of entries in this material from 50 to 34 and retest in multiple locations during the 2014 growing season.

We continue to work on new generations of materials making crosses between these materials and our multiple disease resistant breeding lines as well as our leaf spot resistant materials.

We have identified germplasm lines from the mini-core collection with superior drought and heat tolerance as compared to the conventionally grown varieties and we have begun crossing these accessions with our elite breeding lines. This work can be greatly enhanced through marker development and the identification of markers for MAS. Every selection will be tested for O/L ratios and all of the progeny arising from crosses with nematode resistant parents will be tested using MAS for homozygous resistance to nematode.

The following information is a list of proposed actions for each group of materials that we are currently focused on in our breeding programs. Each bullet details expected numbers of lines, the purpose of the development of each line, and what stage (yield testing, seed increase, and selection) we will be conducting in 2013.

Drought Tolerance – Dr. Mark Burow Lead.

Analysis of minicore yield data. Yield data on the U.S. peanut minicore collection have been completed and analyzed on all 4 tests (2 years x 2 locations, 112 entries x 3 replications x 3 irrigation treatments), and large differences in yield were found. Most significant is that, several accessions were consistently better than checks in three of four trials (Table 2). (The fourth trial suffered from major insect losses in storage and results are not considered to be as reliable). Accessions with the best scores from field measures taken previously tended to yield the best also, meaning that favorable field responses translated into better yield.

Table 2. Yields (lb/ac) of Selected Minicore Accessions under Drought Stress

	Accession	Test 1		Test 2		Test 3		Mean Yield (lb/ac)	Yield Relative to Tamrun OL02
Top 5	COC342	5161	a	2804	a-e	1940	a-i	3302	126%
	COC678	5157	a	2708	a-i	1829	a-o	3232	123%
	COC230	4318	a-h	2871	a-c	2235	a	3141	120%
	COC805	4418	a-f	2814	a-d	2144	a-d	3125	119%
	COC650	4628	a-c	2718	a-h	1652	d-x	2999	114%
Checks	Tamspan 90	3886	b-p	2631	a-l	1868	a-m	2795	106%
	Tamrun OL02	3677	b-w	2511	a-o	1681	c-w	2623	100%
	ICGV 87157	3468	d-C	2430	a-r	1630	e-x	2509	96%
	ICGS-76	2640	t-F	2415	a-r	1937	a-j	2331	89%
Bottom 4	COC367	1783	EF	1122	C-I	1431	j-D	1445	55%
	COC155	2344	B-F	823	G-I	1059	z-H	1409	54%
	COC526	1993	D-F	799	I	1176	w-G	1323	50%
	COC166	1652	F	857	G-I	598	H	1035	39%

- Water use efficiency of minicore accessions. An experiment to measure water use efficiency in pots in a rainout shelter directly suffered from poor seed quality, a major armyworm infestation, and damage to the plants from pesticide application. Seed were increased in the greenhouse over the winter to provide fresh seed for the experiment. Experiments will be repeated in 2014.

- Second year of runner drought yield testing. Tests were performed on selections of a new population of 70 lines for drought tolerance. Large differences in the population were observed, but several lines fared significantly better than the checks (Table 3). Materials were watered approx. once per month, and thus were under significant water deficit stress, which can be seen in the low yields and grades. What is important is that yields of the top lines were significantly higher than Tamrun OL02, but were similar statistically to COC230, which was one of the drought tolerant

accessions identified in the minicore collection. The experiment will be repeated in 2014.

Table 3. Top Performing Runner-type Breeding Lines Developed for Drought Tolerance, tested in 2013.

Genotype	Yield (lb/ac)	Shellout (%TSMK)	Seed Weight (g/100 SMK)
TXL100225-03	2325 a-c	66.5 j-n	56.5 q-t
TXL100225-01	2287 a-d	72.6 a-i	65.5 a-n
TXL100212-03	2166 a-f	68 e-m	61.1 m-s
TXL100225-14	2157 a-f	67.7 f-m	55.3 st
C76-16	2113 a-g	67.3 g-n	69.4 a-d
COC230	1854 a-o	63 m-o	55.3 st
TXL100212-06	1803 a-o	67.1 h-n	52.9 t
TXL100225-05	1722 a-o	63.7 l-o	59.1 o-s
TXL100225-06	1683 a-o	69.9 b-k	63.1 e-o
TXL100212-05	1565 b-p	67.1 h-n	64.6 b-o
TXL100212-02	1563 b-p	60.2 o	56.8 p-t
TamrunOL02	1501 e-p	66.6 i-n	61.1 m-s

- Increase of runner drought populations. Runner minicore accessions having good drought tolerance were crossed with elite, high oleic, multiple disease resistant or early-maturing breeding lines in Lubbock and College Station in 2011 and 2012. Progeny were space planted in 2013, some under limited irrigation, and some under full irrigation for increase. Some of these were used for validating association mapping-derived markers with yield. We plan to advance some of these materials for increase as plant rows in 2014.

Drought Spanish crosses. New crosses were made between Spanish-type drought resistant accessions and the project's elite Spanish cultivars. This involved the new Schubert variety and accessions having improved tolerance to drought or salinity, or earlier maturity. The F_1 generation was obtained in 2013, and these materials will be increased in 2014. Additional crosses will be made to increase the number of progeny. **Heat tolerance screening– Mark Burow Lead**

We previously did testing of the minicore collection for heat stress tolerance, and need to repeat and verify the results because of the newness of the methods used. We found that many varieties are highly susceptible to heat, but several minicore accessions could survive and grow in temperatures up to 50° C (122 ° F). Given the extreme temperatures of 2011, this would be useful to have peanuts that are tolerant of high temperatures. Field

measurements are unpredictable because we cannot control temperature, so measurements were made under controlled conditions in growth chambers.

Experiments were repeated in 2013, but we were not satisfied with the results, because the old seed resulted in slow germination of some accessions, and meant running the heat stress tests on materials at different stages of development. This made it difficult to make meaningful comparisons. Because of this, seed were planted in the greenhouse and increased over the winter of 2013/2014, and have been harvested for repetition of trials in 2014.

Seedling Disease Screening- Michael Baring Lead

The project noted high variability amongst breeding lines for levels of resistance to seedling diseases (specifically Rhizoctonia) at one of our South Texas nursery locations in 2012. This particular test had commercial varieties as well as breeding lines from other major Universities. Only data from the commercial varieties and this programs breeding lines are shown in Table 4 due to the proprietary nature of the other program's breeding lines.

Table 4. Percent Emergence Three Weeks after Planting in South Texas

Entry	%Emergence
Tamrun OL07	62.5a
Tamrun OL11	60.1ab
Tx071304	54.0bc
Tx071305	50.5c
NC-7	40.0d
Georgia 09B	14.3gh
Florida 07	13.0gh
Mean	36.6
p-value	≤0.0001

Tamrun OL07 and Tamrun OL11 both released by this project had emergence better than 60% and since the test was planted at a rate of 5 sd/ft, there were no visible skips in the plots. The two breeding lines Tx071304 and Tx071305 were both above 50% emergence and the plot rows had no skips at harvest. Georgia 09B and Florida 07 had significant skips that never recovered.

Our plan for 2013 was to test several hundred breeding lines using replicated sets and inoculating them with Rhizoctonia in a controlled greenhouse environment during the winter months. This did not occur on time due to several factors including poor inoculum and the loss of two field technicians. We did not see any seedling disease symptoms at any of our field nurseries in 2013 even though we used untreated seed to plant our test plots. We have begun the greenhouse experiments using approximately 100 of our elite breeding lines and 5 commercial varieties. The initial screening is being conducted at Lubbock under Dr. Jason Woodward's direction. These screenings will be replicated under greenhouse control and once we have identified lines with the best level of resistance, we will move to field screenings at Lubbock, Yoakum, and Stephenville, Texas.

Leafspot and Screening

We selected 7 advanced breeding lines that were developed for leaf spot resistance. These lines had various combinations of high yield, high grade, high oleic, good Sclerotinia resistance and good leafspot resistance. None of the seven lines had all of these traits. We intercrossed these seven lines in the summer of 2012. We grew out the F1 progeny during the winter of 2013 just as we had indicated in our 2013 NPB proposal. However, the F1 progeny did not yield as many F2 seeds as we had anticipated. Two of the largest F1 plants did not set any pegs. We believe that this could be due to a recombination of the genes that were introgressed from the wild species hybrid that was originally used to transfer the resistance to leaf spot. Rather than risk the minimum number of F2 seeds that we harvested by planting them out in the field (given the past two years of drought), we held on to the seeds and they are currently being increased in the College Station greenhouse. We anticipate planting F2:3 plant rows for leaf spot screening and evaluation in 2015. While we are increasing these individual F2 plants we are making pollinations from these progeny onto our early maturing and multiple disease resistant breeding lines for future selections.

Markers for yield, drought tolerance, and disease resistance – Mark Burow Lead

The US peanut minicore collection is being tested as a source of needed traits and for molecular markers. Previously, we identified markers associated with field measurements of drought tolerance in the peanut minicore collection. This was done using a new method called association mapping. So far, we used this to find markers for traits associated with drought tolerance, namely flowering, chlorophyll content, and leaf closure during the heat of the day. Markers that we identified previously as being associated with field responses to drought stress also were associated with yield (Table 5). This confirms that these markers should have some usefulness in selecting for drought tolerance not only in field response but in yield under drought stress, which is what is needed to be useful to growers.

Table 5. SSR markers associated with multiple drought traits at two or more years or locations.

Marker	Traits (Effects)
TAM206	Leaf closure (+), flowering (-), pod yield (-), harvest index (-), canopy temperature (+)
TAM207	Leaf closure (+), flowering (+), pod yield (+), plant height (-)
TAM1103	Leaf closure (+), plant height (+), yield loss (75 to 25% ET) (-)
TAM36	Leaf closure (+), plant height (-), yield loss (75 to 25% ET) (-), plant width (+), pod yield (+)
TAM202	SPAD (+), flowering (+), pod yield (+), plant height (-), yield loss (75 to 25% ET) (+)
TAM104	Harvest index (-), pod yield (-), plant height (+)

Work will continue in 2014 on associating marker data with field data on resistance to leaf spot, rust, pod rot, and Verticillium wilt.

DNA marker analysis in collaboration with Dr.Andrew Paterson of a 3rd-generation backcross population from an interspecific cross developed by Dr. Simpson and screened for nematode resistance by Dr. Starr, demonstrated that, instead of the one gene for nematode resistance that we have screened for previously in developing markers for nematode resistance, there are at least 8 different genes for nematode resistance. This new result was the product of using an advanced-backcross QTL experimental design. Six of the genes from the wild species were for resistance and two, surprisingly, were for susceptibility. The gene that has been selected for using markers in developing NemaTam and Webb is the strongest gene. But use of others could be beneficial, because pyramiding two or more genes could reduce the chance of virulent (resistant) nematode populations appearing. Alternatively, development of different varieties possessing different nematode resistance genes would allow planting different resistant varieties in case resistant nematodes are found. Figure 1 is a genetic map of peanut chromosomes, with the colored bars at the sides illustrating the locations of the genes for resistance. We expect to use gene sequences obtained in the genomics project to make improved markers for these genes, and to determine whether any are present in NemaTAM or Webb. We are also working on developing an improved version of the current marker, which is unable to distinguish between resistant plants that produce only resistant offspring, and resistant plants that produce both resistant and susceptible offspring.

Figure 1A. Quantitative Trait Locus Markers for Nematode Resistance in Peanut. The different chromosomes (named as LG##) are shown along with the markers used for that chromosome. Bars mark the location of the resistance genes. Blue bars represent genes in the A genome of peanut, bars in red represent genes from the B genome of peanut. The gene used for selection of resistant varieties so far is the one on LG01, between markers R2545 and Vmc3.

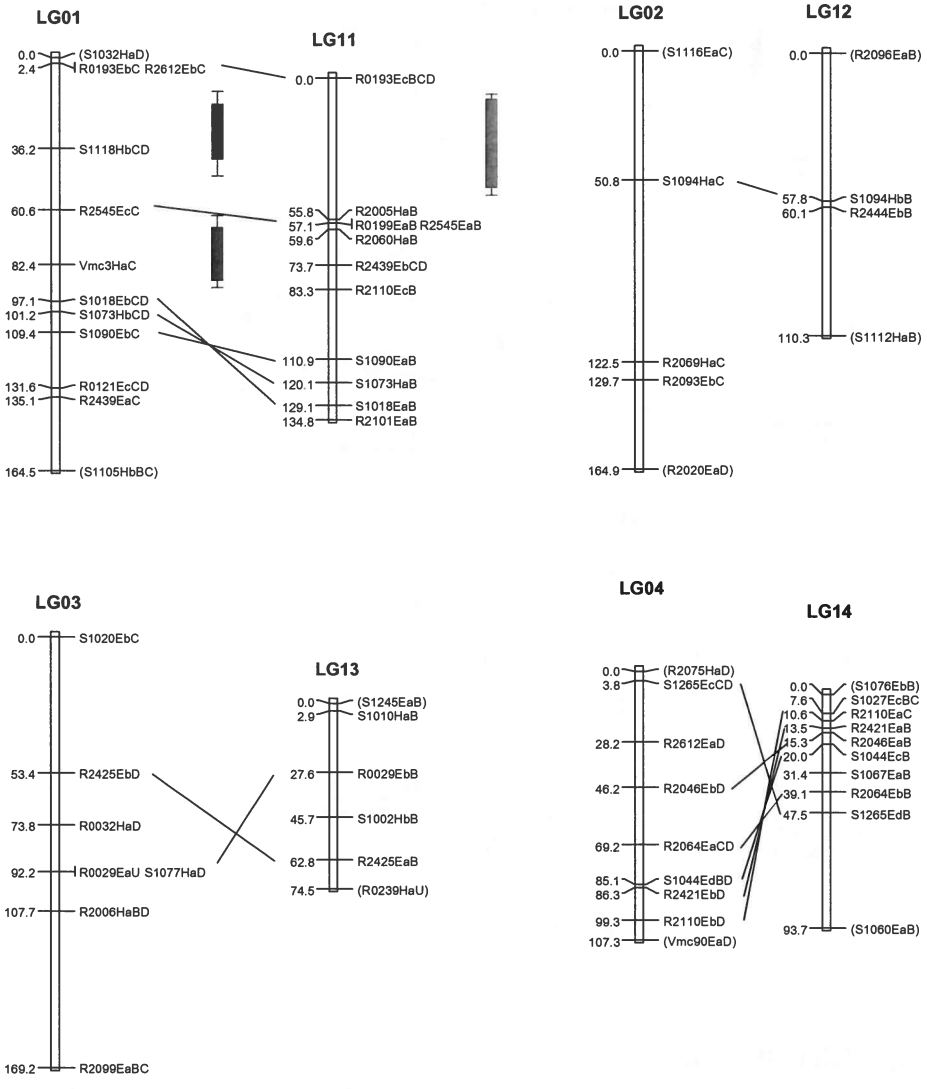
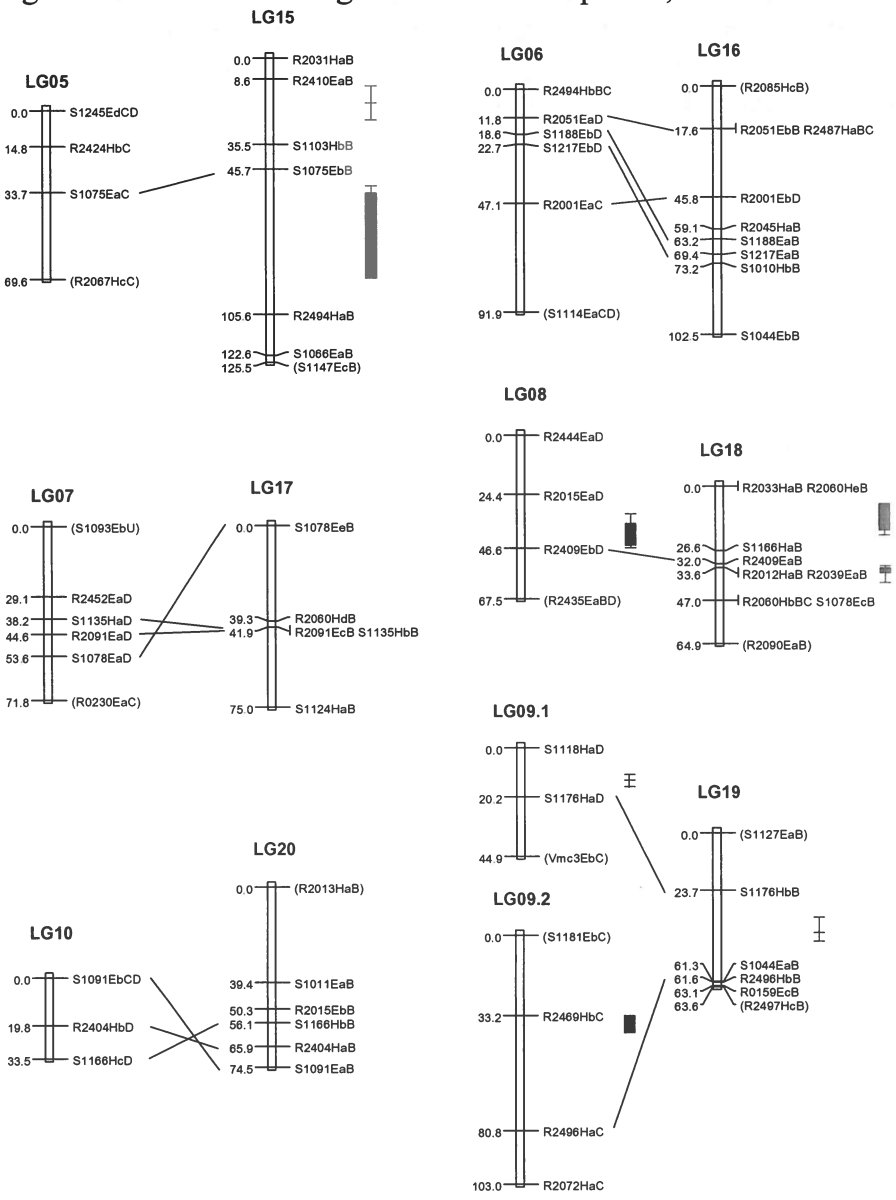


Figure 1B. Quantitative Trait Locus Markers for Nematode Resistance in Peanut. The figure shows the remaining chromosomes in peanut, and additional markers for resistance.



Screening for High Oleic Peanut

- The College Station project screened several hundred seeds from progeny lines which have had a minimum of one year of yield testing and have been selected to go forward into multiple location yield trials. These lines were screened using NIR technology based in Dr. Burow's lab at Lubbock. The Lubbock project re-screened a minimum of 20 seeds from each of its advanced runner and Virginia lines for the high oleic trait, and pure high-oleic seed of all 100 lines were grown out for increase, to assure purity.
- We also screened approximately 30 seeds using Gas Chromatography to make sure that the individual seeds selected were high oleic for crossing purposes.

Quality analyses of advanced breeding materials – Burow, Baring & Simpson

- We screen breeding line samples for flavor, sugar content, fat content, protein content, and blanching characteristics with J.L. Leek and Associates yearly and will continue this to detect any advanced lines that may not be suitable for meeting industry specifications. Based on results from 2013, the Lubbock project has selected 10 advanced early-maturing breeding lines to submit for quality analysis. This data will be used towards making a decision which line to release.