

Final Report for 2018 National Peanut Board funding to the Texas Peanut Producers Board.

I. Subject area: Molecular Genetics & Breeding

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

Funding Year: 2018

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Sub-Project I-1 Multiple Disease Resistant Runner-type trials

The TAMU Peanut project had replicated yield trials located in South Texas, (Pearsall, Dilley, and Derby) and in North West Texas, (Seminole and Seagraves). We conducted 5 Advanced Line Tests (ALT's) across Texas in 2018. These tests included 7 lines developed for drought tolerance, 7 lines developed for Root knot nematode resistance and Sclerotinia resistance, 1 line with high grade potential and Sclerotinia resistance, 1 early maturing line, as well as 4 commercial checks Tamrun OL11, Webb, Georgia 09B, and Georgia 14N. It should be noted that the lines developed for drought tolerance are some of the first generation of crossing completed for the drought project and while they are not the most drought tolerant lines in the project, they appear to have excellent yield potential under normal irrigation to the point that they continue to perform at the top of the tests.

Table 1 on the following page is a combined analysis of all five ALT's across Texas in 2018. The top 7 lines beginning with TXL100212 all came from the drought crossing program and all performed in the a-grouping for yield. In terms of drought tolerance, these lines were not at the top of the test however under full irrigation they have been yielding at the top of the test for the past two years.

Table 1.
Combined Analysis of Five Advanced Line Yield Trials Across Texas in 2018

Entry	Yield in Lbs/A	Grade %TSMK	Seed Size g/100 seeds
TXL100212-03-13	6448a	72.5b-e	69.2ab
TXL100212-05-03	6402ab	71.2e-h	66.6c-e
TXL100212-03-06	6402ab	73.5b	67.8b-d
TXL100212-03-03	6371ab	72.8b-d	68.6a-c
TXL100212-03-08	6292a-c	71.5d-h	64.6e-g
TXL100212-05-09	6273a-c	71.0f-h	68.1a-d
TXL100212-07-08	5981a-d	71.9c-g	63.4fg
TX121082	5947a-d	72.0c-g	62.2gh
TX144370	5914b-e	73.5b	64.6e-g
TX144367	5898b-e	73.5b	64.7e-g
TX144507	5787c-e	71.1f-h	59.1i
TX144506	5757d-f	71.0f-h	55.9j
TX144485	5754d-f	72.9b-c	70.1a
Webb	5736d-f	70.5h	69.4ab
Georgia 09B	5670d-g	73.5b	62.9g
TX144342	5647d-h	72.3b-f	69.0a-c
Tamrun OL11	5416e-h	73.7b	65.6d-f
TXL080243-06	5269f-h	73.2bc	65.6d-f
Georgia 14N	5194gh	76.6a	59.9hi
TX144521	5149h	70.8g-h	66.8c-e
Mean	5865	72.4	65.2
LSD	511	1.4	2.6
p≤0.05	≤0.001	≤0.001	≤0.001

Breeding line Tx121082 has been submitted to the TAMU Plant Release Committee for release as ‘AG18’. It performed in the top statistical grouping again in 2018 with a yield of 5947 across all test sites. AG18 was released to replace Tamrun OL11 because it has a slightly smaller seed size, maintains the same high grade potential, and has a more stable yield across environments and years.

The TX144300’s and TX144400’s were developed for resistance to Root knot nematodes and Sclerotinia. While they performed lower in yield to the drought lines mentioned above, the TX144370 and TX144342 lines have performed well in South Texas which is where they were developed to give growers a nematode resistant line with better characteristics than the previously released Webb variety. These two lines have yielded from 400-600 lbs/a better than Webb and graded 1-3 percentage points higher. Additionally they have a much shorter growth habit than Webb and a slightly smaller seed size in most of the trials over the past three years. We are evaluating the past three years of data and awaiting the 2019 data to make a decision on releasing one or both of these lines.

Sub-Project I-2 Initial Drought Tolerant Runner-type yield trials

We conducted three runner-type drought trials again in 2018 which was the second year of replicated trials for this material. These lines were tested in South Texas near Pearsall under full irrigation and in West Texas under reduced irrigation. The Drought #1 Trial consisted of 23 breeding lines plus the donor parent for the drought tolerance trait (ICGV-76) and the elite parent which was the commercial line Tamrun OL11. To conserve space the data for this test is presented in an abbreviated table showing only the top performing lines of the test (**Table 2**).

Table 2. Drought #1 Yield Trial south of Pearsall, Texas in 2018

Entry	Lbs/a	%TSMK	g/100 seed
TXL100212-03-10	6228a	71.0b-f	62.9a
TXL100212-03-09	5919ab	69.9d-g	62.0a-c
TXL100212-02-05	5881ab	70.7c-g	62.7ab
TXL100212-05-02	5827a-c	73.0a-d	61.3a-d
TXL100212-03-06	5761a-c	74.6a-c	60.8a-e
TXL100212-03-08	5735a-c	70.0d-g	57.9b-h
TXL100212-03-11	5718a-c	73.3a-d	60.0a-e
TXL100212-03-03	5644bc	75.4ab	62.9a
TXL100212-03-13	5619b-d	72.8a-e	60.5a-e
TXL100212-05-03	5589b-d	74.8a-c	62.1a-c
Tamrun OL11	4507i-l	70.0d-g	59.5a-f
Mean	5610	69.1	57.6
LSD	554	3.9	4.9
p\leq0.05	\leq 0.0001	\leq 0.0001	\leq 0.0001

TXL100212-03-10 had the highest yield in the test at 6228 lbs/a. Six other breeding lines performed in the top statistical grouping for yield as well. Many of the lines in the test performed superior to the parent Tamrun OL11 which only yielded 4507 lbs/a. While yields were high at this location, grades for this location are normally mid to upper 70's, but were lower in 2018 with a mean grade of 69.1%. TXL100212-03-03 had the highest grade out of the top yielding lines with a grade of 75.4%. TXL100212-03-06, TXL100212-03-08, TXL100212-03-03, and TXL100223-03-13 were also previously mentioned in the ALT (Table 1). These lines performed so well in 2017 that they were added to the multiple location ALT in 2018 to test them over multiple environments. Many of the top performing lines were moved forward for the 2019 testing.

Drought #2

There were 23 breeding lines and two parents (ICGV-76 & Tamrun OL11) in the Drought #2 Yield Trial. To conserve space data only from the top performing lines and the commercial check are shown in an abbreviated table (Table 3). TXL100225-03-04 had the highest yield at 6186 lbs/a. There were twelve breeding lines that performed in the top grouping for yield ranging from 5350 lbs/a to 6186 lbs/a. The commercial check Tamrun OL11 performed towards the bottom of the test with a yield of 4507 lbs/a. As mentioned earlier in the text, grades were lower than expected at this location. Tamrun OL11 typically grades upper 70's at this site and as seen in Table 3 it graded 66.7 %TSMK. TXL100225-03-08 had the highest grade at 72.1%. The average seed size for the lines in Drought #2 were smaller than Drought #1 with means of 57.6 g/100 seed and 65.2 g/100 seed respectively.

Table 3. Drought #2 Yield Trial south of Pearsall, Texas in 2018

Entry	Lbs/a	%TSMK	g/100 seed
TXL100225-03-04	6186a	70.5a-d	51.6 NS
TXL100225-03-12	6012ab	67.8a-h	53.1
TXL100225-03-07	5896a-c	67.2b-h	52.1
TXL100225-03-06	5890a-c	67.3b-h	55.6
TXL100225-03-10	5888a-c	67.0b-h	53.5
TXL100225-03-13	5808a-d	68.6a-h	52.8
TXL100212-07-05	5741a-d	67.7a-h	57.6
TXL100212-07-12	5585a-e	66.3d-h	57.8
TXL100225-03-02	5520a-f	68.7a-h	55.6
TXL100225-03-08	5466a-f	72.1a	65.7
TXL100212-07-03	5364a-g	67.9a-h	55.8
TXL100212-07-08	5350a-g	69.2a-f	56.6
Tamrun OL11	4600h-i	66.7c-h	57.6
Mean	5256	68.2	54.8
LSD	960	4.4	8.1
p ≤ 0.05	≤ 0.0001	≤ 0.026	≤ 0.1

Drought #3 Yield Test

The Drought #3 yield trial had eighteen breeding lines and the two parents as check varieties. Eleven of the eighteen breeding lines performed in the top grouping for yield with TXL100225-05-02 having the highest yield at 5885 lbs/a (Table 4). Grades for this test were even lower than the Drought #1 & 2 with TXL100225-06-08 having the highest grade at 72.6%. These lines have not performed as well as the lines from the other two tests in south Texas under full irrigation over the past two years.

Table 4. Drought #3 Yield Trial south of Pearsall, Texas in 2018

Entry	Lbs/a	%TSMK	g/100 seed
TXL100225-05-02	5885a	64.0h	57.8b-f
TXL100225-06-07	5729ab	69.1b-f	60.8a-c
TXL100225-05-11	568ab	70.7a-c	60.3a-c
TXL100225-06-11	5664ab	70.4a-d	61.4ab
TXL100225-06-03	5630ab	67.2d-h	53.4gh
TXL100225-05-01	5543a-c	70.1a-d	57.5b-g
TXL100225-06-05	5540a-c	68.2b-f	60.3a-c
TXL100225-06-12	5472a-d	64.9gh	57.8b-f
TXL100225-05-10	5315a-e	66.5f-h	55.3d-h
TXL100225-07-04	5228a-e	66.4f-h	58.5a-e
TXL100225-06-08	5215a-e	72.6a	59.4a-d
Tamrun OL11	5137b-e	68.8b-f	59.3a-e
Mean	5250	68.5	57.7
LSD	741	3.3	4.3
p ≤ 0.05	≤ 0.006	≤ 0.0008	≤ 0.0002

Spanish-type Yield Trials

We conducted advanced Spanish-type yield trials at two locations in West Texas during the 2018 growing season. The tests consisted of 17 advanced Spanish breeding lines and three commercial checks; OLin, Schubert, and Tamnut OL06. As stated above, to conserve space only the top performing lines are shown in the following table.

Six breeding lines and two checks, OLin, and Schubert performed in the top grouping for yield with TXL076236-04 having the highest numerical yield at 5,000 lbs/a (Table 5). The top two yielding lines TXL076236-04 and TXL076225-28 also graded significantly higher than the commercial checks with grades of 76.4 and 76.2 respectively. TXL076236-04 had a slightly larger seed size than the commercial checks at 53.2 g/100 seed, but TXL076225-28 was not different than in size than OLin or Schubert.

Table 5. Combined Analysis of Advanced Spanish-type Yield Trials in West Texas 2018

Entry	Lbs/a	%TSMK	g/100 seed
TXL076236-04	5000a	76.4ab	53.2bc
TXL076225-28	4711ab	76.2ab	48.2f-j
TXL076225-48	4699ab	75.7a-c	47.1i-k
TXL076226-18	4678ab	75.3bc	51.3c-e
OLin	4642ab	74.8cd	50.1d-f
Schubert	4521a-c	72.9f	49.4e-h
TXL076221-06	4483a-d	76.2ab	47.6g-j
TXL076224-08	4473a-d	74.6c-e	49.8d-g
TXL076221-34	4417a-d	75.5bc	47.3h-k
Mean	4267	74.8	49.7
LSD	668	1.4	2.3
p≤0.05	≤0.0001	≤0.0001	≤0.0001

Runner release documents.

We have submitted proposals for release of two early-maturing runner varieties, to be named Tamrun OL18L and Tamrun OL19. These yield as well as or better than Tamrun OL11 and Georgia 09B, grade similarly to Georgia 09B. Maturity of both is better than Tamrun OL07, but Tamrun OL18L is similar in maturity to Tamrun OL12, which is 2 weeks earlier than other varieties. Tamrun OL19 is about 1 week earlier than other runners. Tamrun OL18L has a larger seed size than Tamrun OL19, and is similar to Tamrun OL07 and Weeb.

Table 6. Means across locations and years for multi-location trials (2013-2015):

Genotype	Pod Yield	Shellout	Seed Weight	----- Pod Maturity -----	
	lb/ac	% TSMK	g/100 SMK	% Black + Brown	% Black + Brown + Orange
Tamrun OL18L	5536 a	72.3 b-d	74.4 a	19.4 ab	53.4 a
Tamrun OL07	5344 a-d	72.8 bc	72.5 a	4.5 d	32.1 b
Tamrun OL19	5273 a-e	72.3 b-d	69.8 b	12.8 bc	48.4 a
Flavorrunner 458	5166 a-e	73.1 b	60.2 f	--	--
Tamrun OL12	5114 a-e	71.1 d-f	63.7 e	--	--
Tamrun OL11	4956 b-e	74.9 a	66.3 d	22.6 a	55.9 a
Tamnut OL06	4790 de	68.1 g	60.3 f	--	--
p	0.012	0.039		<0.001	0.006
Mean	5638	72.0	66.4	13.5	49.3
LSD	620	1.4	2.0	7.4	10.1
CV	19.9%	3.66%	5.2%	51.9%	20.3%

These are the means of selected accessions in the runner advanced line tests, 2014- 2016, at from 3 to 6 locations per year. In some cases, significance statistics are not given for accessions that were not present all years. Yields are as good as or better than Georgia-09B, TUFRunner 511, and FloRun 107. Grades are also comparable, except that Tamrun OL11 had a higher shellout than all the other entries in the test.

Table 7. Runner advanced line tests, 2014- 2016.

Genotype	--- Pod Yield ---	--- Shellout ---	--- Seed Size ---
	lb/ac	% TSMK	g/100 SMK
Tamrun OL18L	5359 a	73.5 bc	74.8 a
Tamrun OL19	5176 a	73.6 bc	70.2 b
Tufrrunner 511	5081	73.4	75.3
Florunner 107	5137	73.1	67.2
Georgia-09B	5102 ab	74.4 b	66.1 c
Webb	5067 ab	73.1 c	75.6 a
Tamrun OL11	4786 b	76.7 a	68.2 bc
p	0.037	<0.001	<0.001
Mean	5610	73.4	71.0
LSD	386	1.0	3.2
CV	14.4%	2.9%	7.15%

Large-Seeded Runner population.

We have also evaluated a set of breeding lines that has often had larger seed size than most runners. Other programs have released large-seeded runners. We have evaluated 30 breeding lines in this category, to see if any could be released as a high-yielding runner peanut variety. More testing is needed, but several breeding lines were found that either yielded or graded well. The top line this year actually had a seed size similar to most runners. Overall means were low, as the soil was predominantly clay and not as desirable for high yield as are sandier soils.

Table 8. Means for the Large-Seeded Runner Test, Lubbock, 2018.

Genotype\$	Value (\$/ac)	Pod Yield (lb/ac)	Shellout (% TSMK)	Seed Size (g/100 SMK)
TxL090205-29	501 a	2486 a	78.1 a	68.2 h-l
Flavorrunner458	440 ab	2457 a	72.9 a-g	59.8 lm
TxL090108-01	415 a-c	2003 a-d	75.8 ab	81.8 b-e
TxL090108-11	394 a-d	2269 ab	69.4 c-g	69.5 g-k
TxL090108-12	383 b-e	1790 b-f	74.8 a-c	78.7 c-g
NC-7	372 b-e	1587 c-i	74.7 a-c	96.3 a
TXL080256-02	362 b-e	2078 a-c	74.5 a-d	67.7 i-l
TxL090108-28	338 b-f	1582 c-i	72.8 a-g	86.5 bc
TxL090108-26	338 b-f	1859 b-e	73.9 a-e	78.4 c-g
...				
Tam nut O L06	315 c-h	1691 c-i	73.2 a-f	60.6 k-m
...				
Mean	302	1692	71.0	74.8
LSD	117	517	5.9	9.6
CV	19.0%	18.9%	4.1%	6.3%

Continuing Drought Tolerance Trials.

We began testing a different set of materials in previous years, designed to combine tolerance to water deficit, nematode resistance, and the high oleic trait. We received NIFA funding for this project beginning in 2017, and so are continuing the project under that funding source, and data are not presented here except to summarize. Several accessions yielded numerically greater than check varieties over 3 years under drought. However, grades are low, and we expect that we may need to backcross to the better-adapted parent, but use markers to select for drought tolerance.

Drought populations 1,2, and 3 were tested under drought in West Texas in 2018. However, a significant number of pods were lost due to freezing damage, and it is not clear how much of the differences in yields were due to drought tolerance, and how much were due to pod drop. For this reason, the results will not be presented here.

Quality Analysis.

Seed samples were sent to J Leek and Associates for blanchability testing. The following results were obtained:

Table 9. Blanching data.

Accession	Market Class	Blanched %	Rednose %
Tamrun OL18L	runner	91.4	5.4
Tamrun OL19	runner	88.3	5.0
Tamval OL14	Valencia	96.5	2.0
TxL054529-48	Valencia	92.0	4.8
TxL090105-07	Virginia	99.1	0.0
TxL090105-18	Virginia	95.9	0.0
TxL090105-38	Virginia	97.5	1.8
TxL090106-05	Virginia	96.3	3.8
TxL090106-15	Virginia	83.8	14.1
TxL090106-52	Virginia	86.2	11.1
TxL090206-41	Virginia	90.2	8.6
NC-7	Virginia		

The Virginias are candidates for release next year as a high oleic Virginia variety.

Developing Wild Species Pathway for Introgression of Drought Tolerance

Identify Markers for Drought Tolerance in Mini-core

We sent DNA of the minicore collection and additional accessions to the Texas A&M AgriLife Bioinformatics and Genomics Center for RAD-Seq sequencing. We received approx. 45 GB (gigabytes) (compressed) of sequence data back, and sent a graduate student to the National Center for Genome Research in Santa Fe for a 1 month internship in data analysis. She has begun analysis of the data. Preliminary results are as follows:

Table 10. SNPs identified in the US peanut minicore and additional accessions by RADSeq.

Condition	Number of SNPs
SNPs identified across all accession	109,000
SNPs present in high read depth across >100 accessions	8,100

One of the reasons that many of the SNPs fell out after requiring high read depth in >100 accessions is because we included a number of wild species in the samples that we sent out. These will differ a great deal from each other and the cultivated materials in the minicore collection, and so many of the SNPs may be associated with the wild species. But with previous

marker technologies, it was difficult to find more than a few hundred differences in the DNA among plants. Having 8,100 as a starting point for the minicore accession means that we have still found many more differences than we were able to find in the past.

More work is needed for the analysis, but once we have analyzed the differences and grouped them into sets based on similarity in location when mapped to the Tifrunner genome reference sequence that was developed by the Peanut Genome Initiative, we will use the data to find more markers that can be used for breeding for drought tolerance.

Mapping of SNP's in TxAG-6/Florunner Population

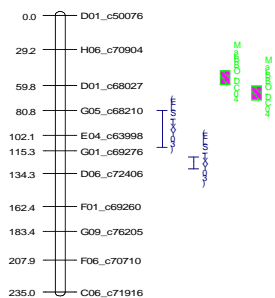
This population was developed from the TxAG-6 interspecific hybrid that contains resistance to nematodes, leafspot, and rust, as well as has some breeding lines with high oil content. We used an earlier version of the map to identify markers for resistance to leaf spots, as well as for yield and maturity.

We worked on refining the map this year, with the goal of mapping the chromosomes (linkage groups) against the DNA sequence of Tifrunner. While many chromosomes matched the Tifrunner well, we consider that two linkage groups, A07 and A09, are under-represented in our map, and we plan to fill in these gaps. This is important, because without these markers, we may miss identifying markers for traits on linkage groups A07 and A09. We plan to go back to the file of sequence polymorphisms to identify additional DNA sequences that we can use to develop primers to use as markers to fill in these gaps.

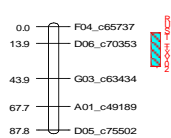
Currently, the map of traits from this population is shown in **Figure NN**.

Figure 1. SNP-based linkage map of the TxAG-6 x Florunner population. Only linkage groups (chromosomes) with markers for traits are shown.

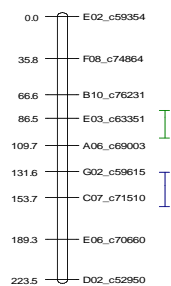
LG10[A01]



LG01[A04]



LG03[A06]



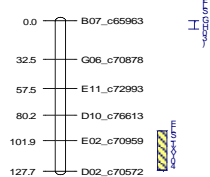
LG04[A08]



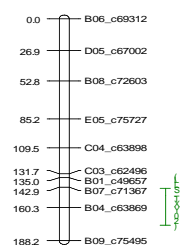
LG06[A01]



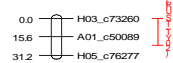
LG12[A09]



LG13[A10]



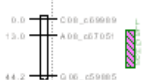
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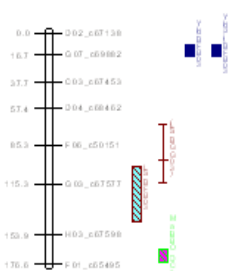
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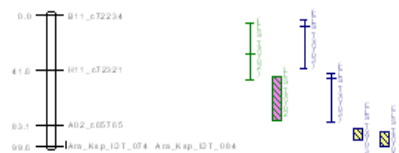
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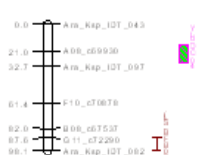
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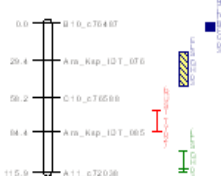
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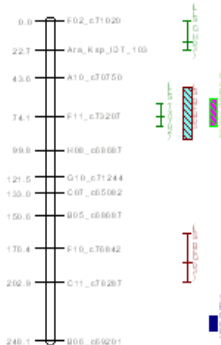
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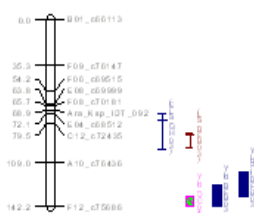
LG08[B06]



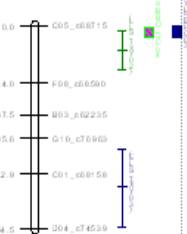
LG15[B05]



LG16[B09]



LG18



Here is a table with the number of markers for different traits in this population.

Table 11. Numbers of SNP markers identified in the TxAG-6 x Florunner population.

TraitFrequency	
ELS	10
ILS	6
LS(unspecified)	5
Rst	4
Yield	9
Maturity	5

Leafspot Resistant Spanish-types

We increased a population BC3-43-09-03-02 X Schubert to introduce leafspot resistance into Spanish peanuts, but because yield was low, this has been planted again for increase again in 2019.

Markers for Leafspot Resistance

Several microsatellite (Simple Sequence Repeat, SSR) markers were identified previously for resistance to early leafspot, late leafspot, or rust in the BC3 introgression population derived from the TxAG-6 x Florunner cross.

We made crosses between two BC3 lines and two Spanish varieties (Schubert and TS32-1) previously. These have been increased in Texas, markers were tested against leafspot scores obtained in previous years at Yoakum and in Ghana. The Spanish introgression populations were sent to Ghana for testing under USAID funding. The results are presented here (if the p-value is less than 0.05, it means that the marker is associated with the trait):

Table 12. Microsatellite markers validated in Spanish Introgression Populations.

Population	Trait	Primer	p-value
TS32-1 x BC3-43-09-03-02	Leafspot 54 DAP 2017	PM3	0.004
“	Leafspot 100 DAP 2018	PM3	0.005
Schubert x BC3-60-02-03-02	Leafspot 110 DAP 2017	PM3	0.041
“	Pod number 2017	PMc348	0.002
“	Leafspot 2018	PMc348	0.04

We expect that the markers will be useful in Texas also. If funding permits, we plan to grow the population that has Schubert as parent in Yoakum in 2020 and test for resistance to leafspot. As this population is segregating a lot of runner plants, we will need to select for the Spanish market type, and may need to backcross to Schubert to recover the desired Spanish type characteristics, using markers to select for leafspot resistance.

Sub-Project IV. SNP Marker Development

Development of a B Genome Mapping Population

This effort is being conducted with other funding but will be important to our efforts in the variety development program when it is completed. We are in the third generation of developing a B-genome mapping population involving two diploid (20 chromosome) wild species. The third generation (F₃) is actually the second generation of single seed descent which we are conducting. We will carry this project two more generations and then establish the map which will be integrated with the A-genome map and then be used for "prescription breeding."

We have cut ends off the seeds of each generation, and the remainder of each seed has been planted for increase. The cut seed ends will be used for DNA extraction.

Screening for Root Knot Nematode Resistance

A total of 904 seeds representing 94 breeding lines were screened by SNP markers to identify lines with RKN resistance. From this screening, forty single plant selections from Population 1A (a population developed to combine the high oleic trait, resistance to drought, and root-knot nematodes) are currently being increased in the field. This was done to purify the breeding lines, to verify that they had nematode resistance, and to purify them for markers for drought tolerance, as they were segregating for markers for drought tolerance.

A related population, called Population 1B, was also screened, and 40 F₂ breeding lines from Population 1B are being increased from which single plant selections will be made prior to planting in 2020. Additional screening is needed, because if we select for seeds that have both copies of each marker allele for all three traits, almost no seeds will remain. We need to select for presence of at least one allele copy for each marker first, allow these seeds to grow and self-pollinate, then select for having both desired forms of each marker in the second round of screening.

There are additional Population 1A and 1B lines available which lack RKN resistance that can be screened with the drought tolerance markers at a later date.

Screening for High O/L Trait

As part of our continuing efforts to develop high-oleic varieties and maintain purity of breeding lines and TAMU varieties, 7,073 seeds were tested for the high oleic trait in the past 12 months (July 2018-June 2019).

The same 904 seeds screened for RKN resistance were also screened for the high-oleic trait by SNP markers. Additionally, 6,169 seeds were screened for high oleic fatty acid content by NIR spectroscopy (see Table 13).

Table 13. Numbers of Seeds screened by NIR for the High Oleic Trait.

Experiment	Seeds Screened
Spanish/Valencia Advanced Line Test	644
Large Seeded Runner	652
Drought F6	859
Spanish High Oleic Increase	757
Valencia High Oleic Increase	481
Runner High Oleic Increase	732
Virginia High Oleic Increase	961
OLin Increase	401
F2 Single Plants	682
Total	6,169