

Title: **Identification of Markers for Maturity and O/L Ratio, Leafspot Resistance, and Resistance to Heat and Drought for Varietal Development**

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Agencies:

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### Objectives.

The proposed research aims to develop markers for cultivated × cultivated crosses to complement the peanut breeding programs in Texas. In this project, we developed markers for drought response in peanut, and are continuing work on markers for leafspot resistance in a wild species introgression population. We also published markers for seed and pod size and weight, and maturity.

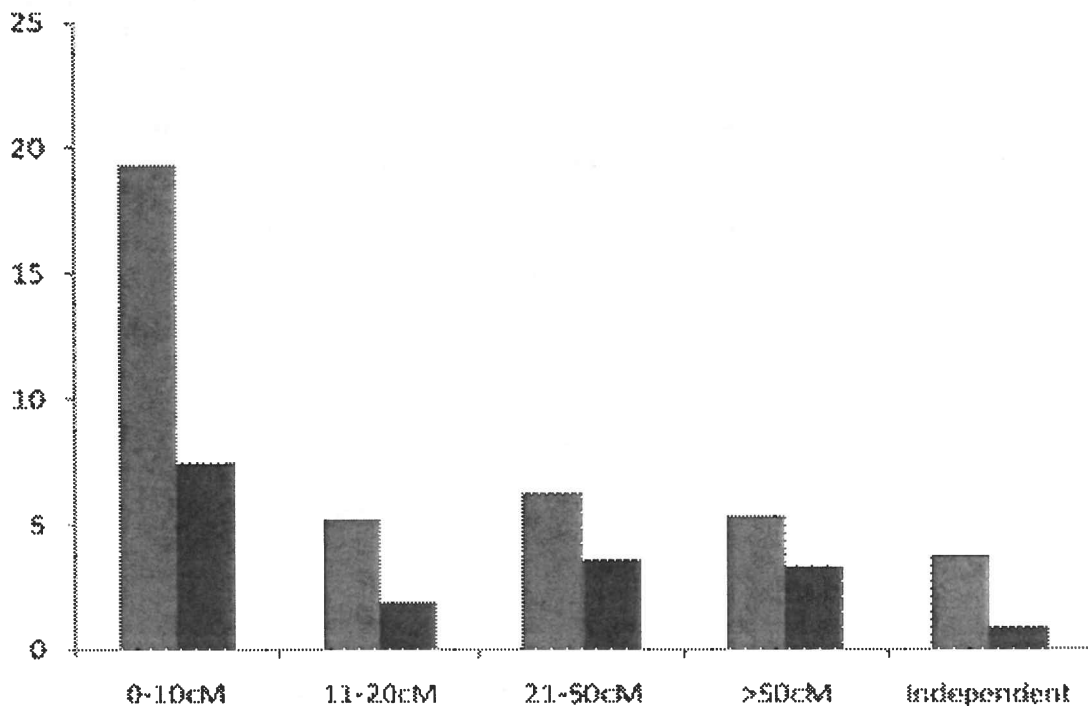
### Results:

The experimental plan was to use microsatellite (SSR, simple-sequence repeat) markers to find differences that can be used for DNA fingerprints to identify plants that have specific traits.

**(1) Publication of a manuscript on identification of microsatellite DNA markers for pod and seed-related traits.** In this, we reported identifying markers for maturity and for pod and seed size.

**(2) Identification of the extent of linkage disequilibrium in peanut.** This is an attempt to find markers in a new way and to identify new and different sources of traits. Instead of needing to make crosses, we tried to determine whether there is significant linkage disequilibrium between genes. What this means is that it would be possible to screen a germplasm collection, such as the US peanut minicore collection with markers, and identify markers to traits in the collection. This would also make it possible to tell whether different accessions have different genes for a trait. If so, then the different accessions could be crossed to combine genes for a stronger effect.

We screened the US peanut minicore collection with markers for 32 mapped positions, chosen to represent all peanut chromosomes. We determined that, unlike in corn where linkage disequilibrium does not extend far, it extends in some cases to significant proportions of a peanut chromosome. Among markers < 10 cm apart, 18% of the markers were in linkage disequilibrium. This means that linkage disequilibrium can be used to identify markers in a germplasm collection. (See Figure 1).



**Figure 1. Comparison of % of SSR locus pairs in LD (at  $p = 0.01$ ) in the entire population and the *fastigiata* subgroup.** Blue bars represent entire population and red bars represent the *fastigiata* subgroup. No data are presented for the subsp. *hypogaea* group because the number of markers was considered to be insufficient to make an accurate determination.

**(3) Association mapping of drought-associated response in the US peanut minicore germplasm collection.** We screened the minicore collection to identify accessions that have drought tolerance. Some of the data were reported in the TPPB Breeding and Testing report. We have found that minicore and other accessions have field responses that indicate significantly better adaptation to drought stress than is present in current peanut cultivars. Field responses included SPAD chlorophyll content (a measure of water use efficiency), flowering, leaf closure during the heat of the day to conserve water, and canopy temperature.

We found markers to the first three of these traits, using the 32 markers tested for linkage disequilibrium. Markers associated with SPAD chlorophyll content are presented in **Table 1**. As association mapping is still a changing science, there are different statistical thresholds used. We consider that markers either having an *SB* suffix or present in multiple tests are reliable. In the case of SPAD chlorophyll, one marker was identified at the most-stringent statistical level (so stringent that it is expected to drop some significant markers), and this and three other markers were identified multiple times, providing strong evidence of their association with this trait. Markers were also identified for flowering and leaf closure. We hope to extend this analysis to finding markers for yield after we have finished analysis of yield in the field experiment.

A larger set of 72 markers screened on the minicore needs to be added to the analysis yet, and these will give us more power to find markers for drought tolerance.

**Table 1: SSR markers associated with soil plant analysis development (SPAD) chlorophyll meter reading (SCMR)**

**Texas Tech Experimental Farm, Lubbock, TX, 2007**

ET replacement	Marker	p-value	R <sup>2</sup> -value
75%	TAM0809_150	0.0045	0.0624
50%	TAM07_182	0.0013S	0.1030
	TA2M06_231	0.0017S	0.0926
	TA2M06_227	0.0059	0.0725
25%	TAM04_241	0.0023S	0.0891
	TAM348_239	0.0043	0.0730
	TA2M02_214	0.0094	0.0697
	TAM348_233	0.0092	0.0613

**J. Leek Farm, Brownfield, TX, 2007**

ET replacement	Marker	p-value	R <sup>2</sup> -value
75%	TAM0809_147	0.0011S	0.0965
	TA2M06_227	0.0031S	0.0934
	TA2M06_231	0.0035	0.0913
	TAM348_239	0.0066	0.0708
50%	TAM0709_257	0.0092	0.0452
25%	TAM04_241	0.0014S	0.0987
	TAM348_239	0.0046	0.0830
	TAM09_126	0.0099	0.0761

**Texas Tech Experimental Farm, Lubbock, TX, 2008**

ET replacement	Marker	p-value	R <sup>2</sup> -value
75%	No significant associations		
50%	No significant associations		
25%	No significant associations		

**J. Leek Farm, Brownfield, TX, 2008**

ET replacement	Marker	p-value	R <sup>2</sup> -value
75%	TAM348_239	0.00062545SB	0.0999
	TA3M12_216	0.0091	0.0698
	TAM145_192	0.0053	0.0672
	TA4M02_204	0.0088	0.0653
50%	TAM305_265	0.0038	0.0503
	TA202_199	0.0087	0.0498
	TAM04_234	0.0057	0.0480
25%	TAM0809_148	0.0085	0.0526

S  $BF_{min}$  with strong to very strong evidence of association ( $BF_{min} \leq 0.05$ )

B Association supported by Bonferroni correction of  $p < 0.05$

**(4) Markers for leafspot resistance.** We are working on finishing scoring a BC3 introgression population with markers. Once this is done, we will finish the work on identifying markers for early leafspot resistance.

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