Project Plan from FY2014
(Field Years 2015-2016)

Title: Breeding of peanut for growth under water-limited conditions on the Southern High Plains, and combination with improved water management practices.

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Summary/Abstract. Previous efforts by the investigators have demonstrated that the U.S. peanut minicore collection contains peanut materials with superior response to water deficit and heat stress compared to current U.S. peanut varieties. In addition, several DNA markers for response to water deficit stress have been identified.

Populations using some of these materials as parents have been developed, and are available for field testing. In addition, primed acclimation, that is, exposure to water deficit early in development, has been shown to acclimate peanut to water deficit and improve yields. Our hypothesis is that, by combining (1) improved genetic response and (2) improved water management technologies, it will be possible to develop a system for enhanced profitability of production coupled with guidelines for the most-efficient use of water. To test this hypothesis, we will:

(1) Evaluate genetic response in F_2, $F_{2:3}$, and $F_{2:4}$ populations of plants, selecting for superior genotypes.
(2) Extract DNA from F_2 plants, and perform statistical analysis to validate association mapping markers (developed previously under OAP support) in two segregating populations. Results will be tested also on $F_{2:3}$ populations.
(3) Examine the physiological basis for superior genotypes selected from the populations, including traits such as root growth, photosynthetic and water use efficiency, and harvest index.
(4) Test the possibility of combining superior genetics with primed acclimation, to merge both genetics and improved water management strategies.

Project narrative

a) Objective(s). The proposal focuses on developing and evaluating genetic technologies and improved water management strategies in peanut for enhancing the economic viability of the agriculture industry and the vitality of the Southern Ogallala Aquifer Region. Peanut is grown on from 110 to 160 thousand acres in Texas, mostly in West Texas, and has on overall economic value to the region in excess of $1 billion annually. We address sub-objective (1) Develop and evaluate water management strategies and technologies that could reduce water withdrawals for irrigation by 20% in 2020 compared to 2012. Development of improved varieties with improved water use efficiency will mean that peanut will be able to be grown profitably while being grown using less irrigation water. We have recently released two early-maturing varieties, which offer the potential for fewer irrigations, and intend to combine this with improved water use efficiency.

b) Rationale/Literature Review/ Conceptual framework. Previous efforts have identified peanut germplasm with superior response to abiotic stress (Hubick et al., 1986; Wright et al., 1991). The PIs have demonstrated that the U.S. peanut minicore collection (Holbrook and Dong, 2005) contains materials with better tolerance to water deficit and heat stress than current U.S. peanut varieties (Kottapalli et al. 2009; Gomez et al., 2011; Rowland et al., 2012). In addition, several DNA markers for response to water deficit stress have been identified (Belamkar 2010, Belamkar et al., 2011).

Recently, we have extended this analysis to yield, and found that two of these markers are associated with higher yield in the minicore collection under deficit irrigation (**Table 1**).

Table 1. SSR markers associated with multiple drought traits at two or more years or locations.

Marker	Traits (Effects)
TAM202_214	SPAD (+), flowering (+), pod yield (+), plant height (-), yield loss (75 to 25% ET) (+)
TAM204_233	SPAD (+), flowering (+), pod yield (+), row width

When tested in a small segregating population of 84 plants made from a cross to combine nematode resistance and drought tolerance, we were able to confirm that two of the markers were associated with differences in yield (**Table 2**). However, the statistical power of the test was limited because of the small population, and it was not considered possible to test single plants effectively for field phenotypic responses to water deficit stress that were measured in row plots. In addition, larger populations are needed for effective breeding work to combine multiple traits needed for a successful cultivar. These will be corrected in the proposed project.

Table 2. Analysis of variance, comparing differences in yield (g pods/plant) in F_2 plants to DNA marker allele scores. Alleles of markers TAM202 and TAM204 were both found to detect significant differences in pod yield per plant.

Source	Score	N	LS Mean	P
TAM202_214	absent	16	127.6	0.022
	present	68	75.0	
TAM204_233	absent	19	50.8	0.041
	present	65	95.0	

Few breeding programs have capitalized on variability in peanut drought stress, rather have historically concentrated on differences in yield alone (Branch and Kvien, 1992), or associated only root length and visual stress ratings with yield (Rucker et al., 1995; Holbrook et al., 2000). This approach has generally met with a lack off success in releasing improved varieties. Our initial screens have found that a yield-based approach misses germplasm that has components of drought tolerance, but poor yield potential, and suggests that the use of more powerful strategies would be beneficial. Populations of these materials have been developed recently, and are available for field testing.

Primed acclimation is the acclimation of plants to water deficit stress by exposure to water deficit early in development. In addition to conserving water in the early stages of development, it has been shown to enhance yield under later water deficit stress (Rowland et al., 2013).

Our hypothesis is that, by combining (1) improved genetic response and (2) improved water management technologies, it will be possible to develop a system for enhanced profitability of production coupled with guidelines for the most-efficient use of water.

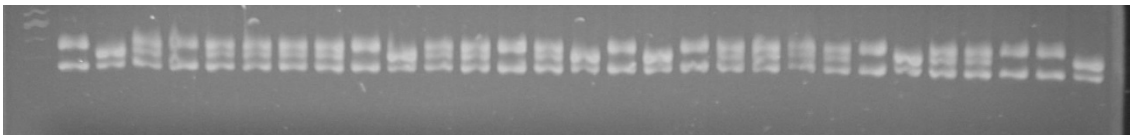
c) How the objective will be met. We will focus in a population developed for superior response to water deficit stress, namely:
(1) Examine genetic response by evaluating F_2, $F_{2:3}$, and $F_{2:4}$ populations of plants, selecting for superior genotypes. The populations to be screened were developed from a cross of Tx071304 and COC270, made to combine drought tolerance and root-knot nematode resistance, and TxL080287 x COC230, made to combine early maturity, and drought tolerance, and the high oleic trait. Early maturity is needed to improve consumer acceptance of flavor, and could save water by reducing the number of irrigations needed. The high oleic trait is desired by industry for improved shelf life, and enhanced consumer health, as a high content of monounsaturated fatty acids has been shown to reduce the incidence of coronary disease.

For F_2 single plants, small chips will be taken off one end of the seed for later DNA extraction and determination of the fatty acid content of the seed; the rest of the seed will be planted. Field testing will be performed initially under water-deficit conditions, because of the large number of accessions (approx. 500) to screen, with selections made for favorable water deficit stress response tested in later generations for yield under full irrigation. Water deficit stress will be performed at 50% or 25% E. T. replacement, as has been done previously for the minicore germplasm screen (Belamkar et al., 2010). Pan evapotranspiration data and relative water content measurements of checks will be used to keep irrigation on schedule; infrared sensors (Mahan et al., 2010) may be used if available. Yield will be measured post-harvest.

For $F_{2:3}$ and $F_{2:4}$ populations (plant rows grown the succeeding generation after F_2 single plants), field scoring will be performed for SPAD chlorophyll content, paraheliotropism, and flowering, and post-harvest testing will be done for yield and grade for $F_{2:3}$ populations, as these were the traits initially identified as responses to water deficit stress, and for which DNA markers were identified. Statistical analysis will be performed on pre-harvest and post-harvest measurements using mixed model methods in SYSTAT or SAS.

(2) DNA Marker Analysis. DNA will be extracted using the Qiagen DNAeasy kit from chips of F_2 seed taken prior to planting. Microsatellite (SSR) markers identified previously under OAP funding (Belamkar, 2010; Belamkar et al., 2011) will be used to amplify markers, which will be scored **(Fig. 1).**

Fig. 1. Primer TAM202 amplification in a segregating population visualized on 4% agarose gel pre-stained with ethidium bromide. The three alleles amplified are 214, 198, and 176 bp. The 176 bp allele is present in all plants. The 214 bp allele is associated with a significant decrease in pod yield.



Statistical analysis will be performed to validate association mapping markers (developed previously under OAP support) in two segregating populations, by analysis of variance. For the F_2 generation, marker scores will be compared to yield by analysis of variance to determine whether there is genetic linkage between the two. For the $F_{2:3}$ generation, F_2 marker scores will be compared to field measurements and yield. Each $F_{2:3}$ line will be considered to be a genetic average equal to the F_2 parent from which the $F_{2:3}$ plants were derived.

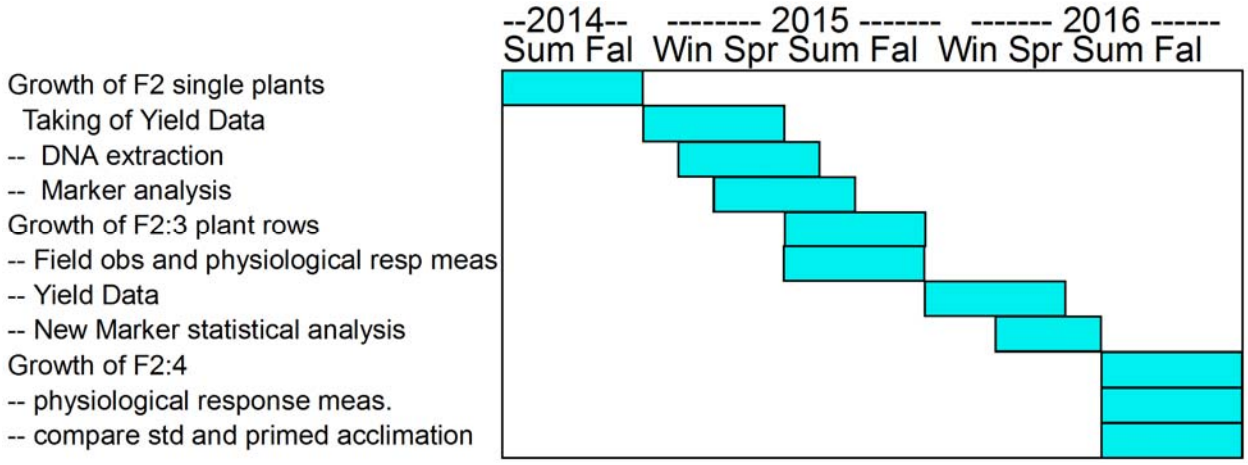
(3) Examine the physiological basis for superior genotypes, including root growth, water use efficiency, photosynthetic efficiency using canopy gas exchange equipment (Baker et al. 2009), and harvest index. This will be done for a limited number of individuals in the $F_{2:3}$ and $F_{2:4}$ generations, due to the intensive nature of the measurements. Selections will be based on the F_2

$F_{2:3}$ data as appropriate, and will be chosen for contrasting responses; check varieties will also be included. Testing will be performed in the growth chamber or field, as appropriate.

For field experiments, two irrigation levels will be established to deliver 3.0 and 1.5 mm of daily irrigation. Irrigation will commence following the first day of water deficit stress using the canopy temperature BIOTIC algorithm for peanut (Mahan et al. 2005). After sowing, rhizotron tubes will be installed at 1m depth, 0 cm (in the row), 40 cm, and 80 cm (furrow) from the planted row. Rhizotron images will be collected and 30, 90, 120, and 150 days after planting. In-season soil moisture will be monitored using watermark sensors and total water use will be determined using gravimetric water content of soil 2 m soil cores at the beginning and end of the season. Additionally, the entire experimental plot will be monitored at for 1 week at 30 (pre-flowering), 90 (peak pod formation), and 120 (pod fill and maturity) days after planting using a thermal imaging camera system and data collection algorithm developed by co-PI Mahan. Root growth profiles will be quantified using procedures described by Rowland et al. (2013). Leaf-level gas-exchange will collected at 30, 90, 120 dpa 24 h prior to irrigation and 48 and 72h following irrigation to quantify water-deficit stress during the irrigation interval and test for differential response to water-deficit and acclimation.

(4) Test the possibility of combining superior genetics with improved water managements practices (primed acclimation), to merge both genetics and improved water management strategies. The best $F_{2:4}$ lines (based on $F_{2:3}$ data) will be grown under the primed acclimation irrigation regime, along with checks. Other treatments will include full irrigation, and water deficit stress. Data to be taken will include yield and water usage. Results of treatments will be compared to determine the most-effective water management strategy.

Timeline.



d) Expected outcomes include:
(a) Selection of superior breeding lines under water deficit stress, that have the potential for release, with the goal of profitable production under limited irrigation.
(b) Validation of association mapping-derived markers. If these can be validated, it would allow selection for water stress tolerance at the F_2 seed level, permitting selection of the most promising materials before beginning field trials, and then allowing intensive evaluation of materials for other traits after pre-selection for response to water deficit stress.
(c) In-depth physiological characterization of water deficit-tolerant lines, in addition to the visual observations made in earlier generations and studies.

(d) Determination whether it is possible to combine improved genetics and water management practices to obtain a synergistic response, saving both water and increasing yields compared to current varieties.

Technology Transfer. Results will be published in refereed scientific journals, and in scientific meetings. Any varieties resulting from this work will be released after review by the Texas Plant Release Committee, as has been done for varieties such as Tamrun OL12 and Schubert (Burow et. al., 2014). Seed will be multiplied under the auspices of the Texas Foundation Seed Service. An economic impact assessment will be developed separately in the future if initial results demonstrate favorable results.

Authors' Related Publications:

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Kottapalli, K. R., R. Rakwal, J. Shibato, G. Burow, D. Tissue, J. Burke, N. Puppala, M. Burow, and P. Payton. (2009) Physiology and proteomics of the water-deficit stress response in three contrasting peanut genotypes. *Plant, Cell, Envir.* 32: 380-407.

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Branch, W. D., and C. K. Kvien. 1992. Peanut Breeding for Drought Resistance. Peanut Sci. 19: 44-46.

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Holbrook CC, and Dong W. 2005. Development and evaluation of a mini core collection for the U.S. peanut germplasm collection. Crop Sci. 45:1540-1544.

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Rucker KS, Kvien CK, Holbrook CC, and Hook JE. 1995. Identification of peanut genotypes with improved drought avoidance traits. Peanut Science 22: 14-18.
Wright, G.C., Hubick, K.T., Farquhar, G.D., 1991. Physiological analysis of peanut cultivar response to timing and duration of drought stress. Aust. J. Agric. Res. 42: 453-470.