Ogallala Aquifer Program
2008 Final Report
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Title: Identification of Mechanisms of Heat Stress Tolerance in Peanut, and Use for Screening a Population for Improved Heat Stress Tolerance.

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Significant Findings:
Several minicore accessions were more heat-tolerant than cultivars. Enhanced respiratory biodemand was correlated with heat injury and sugar content under stress, and pollen fertility was correlated with acquired thermotolerance. There appear to be at least two mechanisms of heat stress tolerance.

Summary:
Recent results from the Economic Assessment and Impact Team of the Ogallala Aquifer Program indicated the development and use of cultivars that use less water can reduce withdraws from the Ogallala Aquifer while enhancing farm and community economic outlook. Contrasting genotypes of the U.S. peanut mini-core collection were evaluated by different measures of heat tolerance by a team of researchers from Texas AgriLife

Research, Texas Tech University and ARS in Lubbock,Texas, and New Mexico State University. This was done to determine whether differences in heat tolerance exist within the cultivated peanut germplasm pool, and whether there are different mechanisms of heat tolerance in peanuts. Several accessions were significantly more heat tolerant than others. There appear to be at least two distinct mechanisms of heat tolerance, suggesting that genetic improvement is possible.

Final Report:

Purpose: Our long-term goal is to develop peanut varieties with tolerance to heat stress. In this project, we are attempting to determine whether different measurements of heat stress response indicate the operation of different mechanisms of adaptation to heat stress. This is important for peanut varietal development, because multiple measures of tolerance will be needed if different assays represent different methods of adaptation to heat stress.

Objectives: The goals of this proposal are (1) to determine whether there are significant differences among different rankings of peanut germplasm, and (2) to use these measures for early-generation selection in a population developed to incorporate improved heat tolerance.

Procedures:
1. Green house experiment. We have previously screened the core subset of the U. S. peanut core germplasm collection by the ERBD assay (Burke, 2007), relative heat injury (membrane leakage), and for total sugar content among the 72 accessions screened under greenhouse conditions.

2. Conviron experiment. A total of 15 genotypes (the most-tolerant and most-susceptible lines according to the result of Experiment 1) from core subset of the US core peanut germplasm including cultivated genotypes were evaluated by multiple methods to determine whether consistent differences are observed among methods adopting RCB design with 3 replication each (control & stress). During the flowering stage, stress treatment plants were transferred to a programmable Conviron chamber to impose chronic heat stress (40 C) for 5 days. The following physiological parameters were recorded at peak heat stress: MFI 3 DAP (days after planting), MFI 5 DAP, number of flowers under stress and recovery, pollen fertility under stress and recovery.

3. Acquired thermotolerance assay. The same 15 genotypes as in (2) were included for this experiment. Ten day-old seedlings were grown in the dark in a growth chamber, with pulsing of light for 2 min every 2 hours to ensure that etiolated seedlings are able to grow. Growing of seedlings under intermittent light regime to enhance leaf expansion without significant chlorophyll accumulation. The leaf bunches were taken from the respective plants and transferred to CELTECH for acclimation treatment 38 C for 4 hr, then exposed to heat shock at 50 C for 30 min, and transferred to a lighted growth chamber for growth at 24 C for 16 hr. Heat shock response was be measured by greening of leaves at 16 hr using a Minolta SPAD chlorophyll meter. The same experiment was repeated second time with seed size to determine whether seed size could affect the apparent heat stress tolerance results.

Results:
Greenhouse experiments to identify sources of heat stress resistance within the minicore collection found

significant differences among accessions by the enhanced respiratory biodemand assay, suggesting the potential utility of the minicore as a source of different genes for tolerance to heat stress. A subset of these accessions has also been characterized to date at seedling and reproductive stages using acquired thermotolerance (ATT) and metabolic fitness index (MFI) measurements, counts of flowers during heat stress and recovery, and number of fertile pollen grains. There are significant differences for heat tolerance in peanut genotypes under stress in this study. The correlation study revealed that ERBD under stress showed significant positive correlation with both relative heat injury (0.757**) and sugar content (0.892***). This clearly indicated that ERBD assay can possibly be used as a proxy for electrolyte leakage and sugar accumulation. There was no strong correlation between the MFI and ATT, indicating differences in the mechanisms of tolerance associated with the measurements. Interestingly pollen fertility under stress showed highly significant positive correlations with acquired thermotolerance (0.649**). There appear to be at least two different mechanisms of tolerance.

Plants were screened for heat shock response by acquired thermotolerance a second time, using seeds of different sizes to test also whether seed size had an effect on acquired thermotolerance. One-quarter of the accessions have been re-tested to date, and the same relationship among individuals has been observed, with one accession having the greatest heat tolerance, and others being intermediate. Although there was a statistically-significant effect of seed size on acquired thermotolerance, the effect was 30-fold smaller than the range of differences among accessions, and was not considered to have had a biologically-meaningful significance.

Based on the present and previous results, the genotypes ICGS76 and ICGV87157 possessed good tolerance to heat stress while Tamrun OL02 and Spanco were susceptible. Based on these results, parents with contrasting heat stress responses and of different market types for greater molecular polymorphism have been selected. Crosses (ICGV87157 × Tamrun OL02, ICGS76 × Tamrun OL02, and ICGS76 × Spanco) were made to develop F_1 hybrids for expansion of current or production of additional F_2 mapping populations. We have increased two F_2 populations for testing of heat shock response, and identification of markers for this trait. A total of 2245 and 2559 F_2 seeds have been obtained for the two populations; this will allow multiple testings for heat stress response and for drought tolerance in the field. Plants of a third population were weak and many died, and therefore this population was not carried forward.

Conclusions and recommendations: It is recommended that acquired thermotolerance measurements be taken on the entire minicore collection. This may give a larger range of responses, and will help with identification of markers for stress tolerance by association mapping. In addition, both chlorophyll fluorescence and acquired thermotolerance measurements be made on experimental populations developed for heat stress response.

Presentations and non-peer reviewed publications:

Burow, M. D., M. G. Selvaraj, K. R. Kottapalli, N. Puppala, P. Payton, D. Porter, and J. J. Burke. (Mar. 2008) Identification of Mechanisms of Heat Stress Tolerance in Peanut, and Use for Screening a Population for Improved Heat Stress Tolerance. Ogallala Aquifer Initiative.

K. R. Kottapalli, R. Rakwal, J. Burke, G. Burow, M. Burow, N. Puppala, P. Payton (Jan. 2009) Physiological and transcript responses to Heat Stress in Peanuts at Reproductive Stage. Poster P658 Plant & Animal

Genome Conference 2009 at San Diego, California.

Peer reviewed publications published in reporting period:

Kottapalli, K. R., M. D. Burow, G. Burow, J. Burke, and N. Puppala. (2007) Characterization of the Core of the U. S. Peanut Core Germplasm Collection using SSR Markers. *Crop Sci.* 47: 1718-1727.