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I. Physiological and genetic evaluation of peanut germplasm for drought tolerance through water deficit exposure during early peanut development

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III. *Report from 2009 NPB funded research.* Field experiments were carried out at Dawson, GA and Lubbock, TX to compare among peanut genotypes their growth and symbiotic nitrogen fixation activity under water-deficit conditions. At Dawson, six peanut genotypes selected from India, genotypes (PI 259747, TMV2, ICG 11376, ICGV 86015, ICVG 86388, ICGV 86388) and four commercial cultivars (York, Georgia Green, Georgia 06G, Florida 07) were studied. The genotypes were grown in duplicate plots under moveable rainout shelters that prevented rain from reaching the peanut plots during rain events. At Lubbock, there was no need for the moveable shelters due to the natural aridity of this location. Therefore, the same ten genotypes plus an additional 11 India genotypes and six commerical cultiars were studied were tested at Lubbock.

The plants were initially grown under well-watered conditions for about 1.5 months until leaf canopy closure was reached for nearly all plots. At this time, water was withheld from the plots to impose drought conditions. An initial harvest of all plots was made to determine mass and nitrogen content at the beginning of the drought period. The plots were then subjected to a three-week period of water deficit. All plots were irrigated with a small amount of water (approximately 10 mm) only when nearly all genotypes were at or near wilting. At the end of the three-week period, the plots were again harvested for mass and nitrogen content.

The mass accumulation and nitrogen accumulation under drought conditions was calculated based on the difference between the two harvest dates. The mass accumulation difference is a direct measure of the ability of the genotypes to sustain growth under water-limited conditions. Similarly, the difference in nitrogen accumulation is an index of sustained symbiotic nitrogen fixation (the plots were not fertilized with nitrogen) under these conditions. Especially, crucial is the ratio between nitrogen accumulation and mass accumulation. This ratio reflects the comparative sensitivity within a genotype of nitrogen fixation activity and growth under drought conditions.

The results from Dawson indiated that superior drought tolerance was exhibited by ICGV 86388 and Georgia Green. The complete data set from Lubbock are not yet available. Once all data are available a manuscript will be prepared reporting these field tests of peanut genotype drought tolerance.

Transpiration efficiency is considered a principal trait to screen drought tolerance in peanut. Increased transpiration efficiency is associated with increased water conservation by restricting transpiration rates under high evaporative conditions without showing impact on gas exchange rates under modest atmospheric vapor pressure deficit (VPD). In greenhouse experimemnt, we investigated the peanut response to transpiration demand imposed by different levels of VPD. These results provide a better understanding of the physiological basis for variation in transpiration efficiency, which could be useful in selecting parental lines in future breeding programs for drought tolerance.

Nine peanut commercial varieties C-99, GA-06-G, Tiffrunner, AP-3, York, Florida 07, GA-04 S, AT-3085 and C-76-16 were selected to test their transpiration response to VPD. Six experiments were carried out in greenhouses at the University of Florida, Gainesville, FL with approximately 30-day-old plants. Instead of a continuous increase in transpiration rate with increasing VPD, all nine genotypes exhibited a breakpoint at an average VPD of 2.5 kPa. Above the breakpoint VPD, these genotypes showed little further increase in their transpiration rate. The stability in transpiration rates at high VPD levels could be an important trait to conserve soil water under water-deficit conditions.

Two dry-down experiments were conducted to measure additional drought traits: transpiration rate, transpiration efficiency (TE), FTSW (Fraction of transpirable soil water)-threshold, and specific leaf area (SLA). These experiments were also conducted in greenhouses at the University of Florida, Gainesville, FL. No significant variation was observed in TE among these varieties under both water-deficit treatment and well-watered conditions. Average FTSW-threshold for stomata closure during the dry-down was 0.4 for all nine varieties and there were no significant differences for this trait. The nine varieties had significant differences ($p<0.01$) in both transpiration rates and SLA under drought. However, transpiration rate measured per leaf area was found to be non-significant for the nine genotypes under both well-watered and water-deficit treatments.

Five peanut genotypes differed in their break point and TE (Devi J.M. et al., 2010) and five commercial varieties were selected to study further for the involvement of leaf hydraulics and aquaporins for transpiration to the VPD response trait.

For the molecular/genetic component, the 12 original cDNA libraries that were subjected to 454 sequencing were compared to each other and to the physiological field responses. Eleven sequences with homology to the following were found to be significant: psa A protein of photosystem I, plasma membrane intrinsit protein 1A (water channel), phosphoribulokinase, ribosomal protein L1 family, phosphate transporter 2, alanine-glyoxylate transaminase, ATPase alpha subunit, chloroplast ribosomal protein L2, glyceraldehyde 3-phosphate dehydrogenase A subunit, and D-fructose-1-6-bisphosphate (FBPase). For the first time, we have physiological measurements that have direct linkages with some of these sequences. Work is now underway to confirm their differential response to drought and determine if their responses are correlative or causative. Additionally, we are exploring the molecular response of aquaporins, since results from the greenhouse point to their involvement.

Reference:

M.J. Devi, T.R. Sinclair and V.Vadez (2010), Genotypic variation in peanut for transpiration response to vapor pressure deficit. Crop Science. 50:191-196.

FINAL PROJECT REPORT
2009 SPRI/NPB

ARS AGREEMENT No. 58-6604-9-227

Title: **Physiological and genetic evaluation of peanut germplasm for drought tolerance through water deficit exposure during early peanut development.**

Investigators: W.H. Faircloth, D. L. Rowland, T. Sinclair, M .Gallo, B. Barbazuk

Objectives: Predictions and forecasts of the accelerating tolls of drought exacted on U.S. agriculture are coming to fruition, and the worsening conditions in the peanut producing regions are no exceptions. Year after ear for the past two decades, drought has devastated peanut yield and grade in at least one of the major peanut producing regions, and recently, the effects are becoming more intense and frequent. Most factions of the industry, from growers, shellers, and manufacturers, are recognizing the importance of developing strategies to sustain peanut production under water deficits. We propose to identify critical physiological and genetic traits that could be used as markers for drought tolerance. Further, we wish to expand our evaluations from tow to a more diverse set of genotype with the goal of developing more drought resistant cultivars. Our specific objectives were: 1) identify variability that exists among peanut genotypes in physiological response to drought stress; and 2) correlate genetic responses with said drought tolerance mechanisms. The latter of the two objectives was funded separately through an agreement with Dr. Gallo.

Methodology: Field plots were established at the National Peanut Research Laboratory in Dawson, GA. The field plots were located with environmental control shelters, which allow for precise irrigation and through an automated roof system, are also protected from rainfall interference. Four 18x40 ft shelters were utilized for the project. Each plot was filled with Tifton series field soil amended (appox. 50%) with a Terrell sand to increase drought potential to a depth of 5 feet. This was the third year of field trials in this soil, therefore no fertilizer was needed, as indicated through a routine UGA soil test. Soil was fumigated with dazomet (Basamid®) prior to peanut planting to eliminate fungal pathogens and reduce weed seed germination.

Four predominant peanut cultivars were selected for screening along with six cultivars from the ICRISAT germplasm collection. These represent dominant breeding lines and have shown drought tolerance potential. Peanut cultivars were: Georgia Green, Georgia-06G, Florida 07, York, ICVG 86388, ICG 11376, TMV 2, ICVG 86015, ICV 87141, and PI 259747. Individual cultivars were planted in 4-row blocks and arranged in a completely random design within the four shelters, with two replications. Peanut were seeded at a rate of 3 seeds/row ft on 24 inch row spacing. Planting date was 8-Jun 2009. At 10 days after seeding, plots were checked for uniformity of stand and additional seed were added to York plots only, due to poor germination. Standard cultural practices were followed regarding pest management and crop production.

From planting until canopy coverage (74 days after planting), peanut were irrigated and/or rainfed at a rate of 1.5 inches/week for maximum development. Beginning at canopy coverage, peanut plants were subjected to drought conditions as follows. Peanuts were allowed to dry down to intermediate stress levels as indicated by mid-day stress (leaf flagging). Subsequently, irrigation was applied at 0.3 inches whenever stress was detected. This equated to irrigation every 3-4 days, depending on temperature. The shelters were automated at this time to prevent rainfall interference. This deficit irrigation strategy was maintained for a three week period until 15-Sep. In effect, the plants were under a continuous moderate drought stress, but not such a severe stress that they began to shut down. Upon completion of the stress period, irrigation and/or rainfall was resumed at 1.5 inches per week until harvest. Plots were hand inverted at optimum maturity and dried. Due to the removal of large quantities of plants for sampling, yield was not estimated. Pods were threshed and saved for future use.

Data collection/sampling procedures: Two days prior to the drought stress period, plants were harvested (H1) as follows. All plants were removed from a 3-ft section of two adjacent rows (12 ft^2) to a depth of 12 inches. The number of plants in that 3-ft section was recorded. Plants were washed carefully and separated into above and below ground portions. Below ground material was further segregated into pods (> 5mm in diameter) and roots. Peg attachments were considered as above ground material. Fresh weights of all materials were recorded and samples then placed in a 60 C dryer for 48 hr. Dried weights were recorded. A subsample of dry material was sent for nutrient analysis. Leaf and root materials (fresh) were also collected and delivered to Univ. of Florida Genetics Institute for further analysis. A second plant harvest was done on 15-Sep (H2) following the same protocol.

Results: Peanut response to drought stress was highly variable among the 10 cultivars tested. Figure 1 illustrates the diversity of growth habit among the cultivars chosen. Notably, three cultivars decreased in root biomass after the drought period: Florida 07, ICVG 86015, and ICVG 87141.

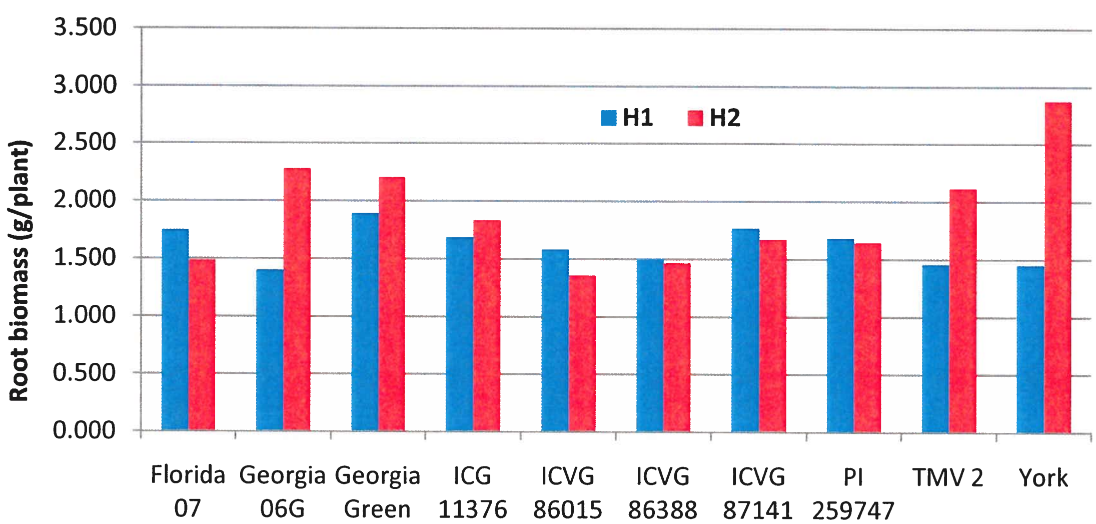

Figure 1. Root biomass before (H1) and after (H2) a 21 d exposure to moderate drought stress.

A decrease in root biomass can indicate poor drought tolerance, depending on development of the peanut plant at the time of stress. To better interpret these results, pod mass per plant must be considered. These same cultivars have the lowest increases in pod mass per plant from H1 to H2 (Figure 2), suggesting that they have decreased efficiency in filling pods under stress. Four cultivars have notably lower pod mass both before and after drought stress those being ICVG 86388, PI259747, TMV 2, and York.

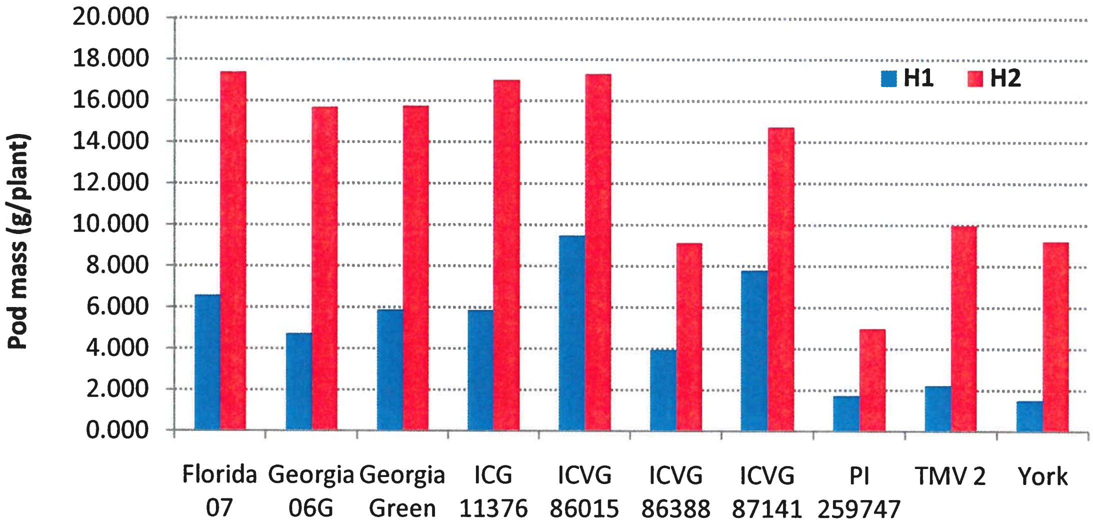

Figure 2. Pod mass before (H1) and after (H2) a 21 d exposure to moderate drought stress.

Leaf biomass prior to drought stress was lowest for Georgia-06G, PI 259747, and York (Figure 3). Of these three, York is known to have a decreased plant size, lending to improved drought tolerance. Interestingly, these same three cultivars showed dramatic increases in leaf biomass during the imposed drought, suggesting some drought adaptation.

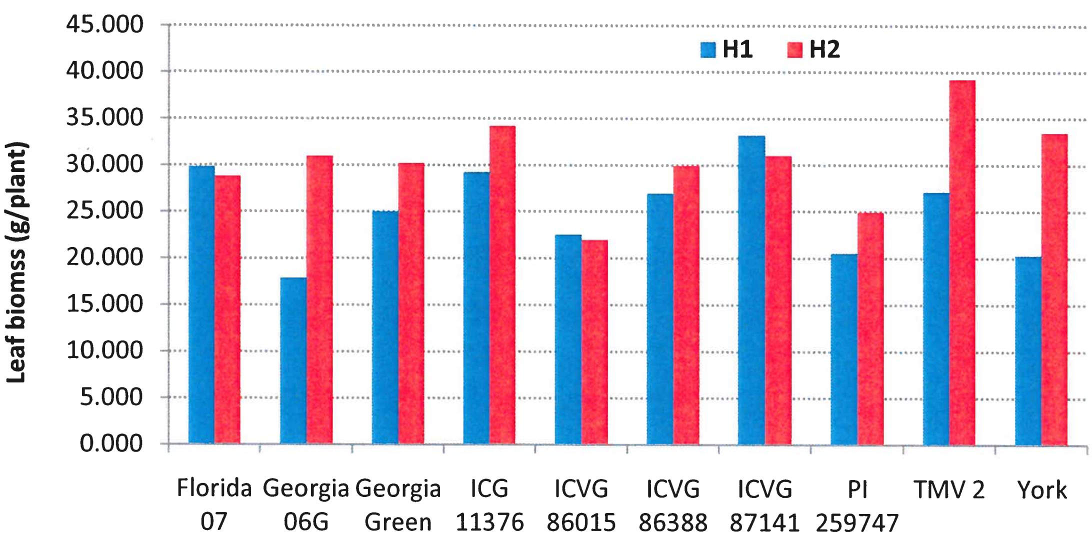

Figure 3. Leaf mass before (H1) and after (H2) a 21 d exposure to moderate drought stress.

Lastly, Sinclair has previously reported that the ability to maintain nitrogen fixation during drought is linked to overall drought resistance. Accordingly, plants were analyzed for N content before and after the drought period. Table 1 suggests, based on Sinclair's reporting, that the following cultivars have adapted drought response: Georgia-06G, Georgia Green, ICVG 86388, TMV2, and York.

Cultivar	H1	H2	% change
	---------*Nitrogen %* ----------		
Florida 07	3.129	2.995	-4
Georgia-06G	2.980	2.918	-2
Georgia Green	2.955	2.891	-2
ICG 11376	3.110	2.851	-8
ICVG 86015	2.919	2.670	-9
ICVG 86388	2.851	2.846	-0
ICVG 87141	3.098	2.785	-10
PI 259747	3.094	2.859	-8
TMV 2	2.834	2.888	+2
York	3.093	3.075	-1

Table 1. Nitrogen content (total of pods, roots, leaves) of ten peanut cultivars

Four of the six ICRISAT peanut cultivars did not maintain nitrogen fixation compared to all other lines, suggesting that these line may not confer drought resistance through breeding. Based on this set of data, several peanut cultivars and breeding germplasm have been identified as candidates for drought tolerance. This work is to be combined with the sister project, lead by Dr. Maria Gallo, Univ. Florida, to evaluate genetic markers that can be used for drought screening in breeding studies.