TITLE: Role of Calcium in Peanut Seed Development

Calcium is the most important element for peanut seed development and viability. Research shows that optimum seed development of peanut varieties with large seed requires more calcium than those with smaller seed. Limited research has shown genetic differences in calcium uptake.

OBJECTIVES:
1) Field Studies: To provide valuable information on the calcium requirement of varieties of different seed size and relative maturity. The ultimate goal is to determine the optimum calcium needed for germination of late maturing cultivars with excellent disease resistance such as C-99R.

2) Lab Studies: To obtain fundamental advancement in our understanding of the calcium signaling network involved in peanut seed developmental. Ultimately, such knowledge will lead to successful manipulation of this network to significantly aid in breeding for improved varieties.

EXECUTIVE SUMMARY:

Large-seeded cultivars are becoming increasingly popular among growers and will likely require more calcium than previous cultivars such as Georgia Green and Florunner, two dominant cultivars over the past 30 years. Application of gypsum to supply calcium for peanuts is costly for growers, but is critical in attaining maximum germination and seedling vigor. Therefore, it is important to understand the differences in calcium requirement between genotypes with large-seed and normal seed. Breeders need basic information on why cultivars differ in calcium requirements and methods to select for types with minimal calcium fertility requirement. This work investigated a class of proteins, CDPK's and will determine how they differ between cultivars. It also showed that the large seeded cultivar C-99R (the basis for seven of the most popular cultivars today) was more sensitive to calcium nutrition than was Georgia Green. This information will be valuable for grower in planning gypsum applications.

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METHODS: Field Studies: A normal seed sized runner, Georgia Green, and large seeded runner, C-99R, were planted on May 20, 2008 at the Plant Science Center in Citra, FL. Treatments consisted of no applied gypsum or two applications of gypsum in a randomized complete block design. There were six replications. Sampling was performed four times during the season with the sampling dates being: August 6 (76 DAP), September 12 (113 DAP), 3 October 3 (133 DAP), and at harvest on October 6. 2008. For all sampling dates, a one meter row was dug and all peanuts from those plants were collected and the following evaluations were made:

1. Seed developmental stage characterization and appearance
2. Pod and seed-specific calcium levels
3. Yield

Lab Studies: It has been well established that Ca^{2+} is an important second messenger in plant signal transduction pathways during growth and development. Consequently, there are calcium sensors that can detect particular calcium signatures and influence downstream effects, such as changes in protein phosphorylation and gene expression. In plants, the most widely investigated Ca^{2+}-binding sensory proteins are calcium-dependent protein kinases (CDPKs). Genome-wide identification of the CDPKs has been conducted in *Arabidopsis* and rice, and there is growing evidence that CDPKs are vital for normal seed development and production. The importance of Ca^{2+}in peanut seed development suggests that CDPKs are logical targets to explore as candidate sensors. From each of the sampling dates above, we collected developing seed, staged them and immediately froze them in liquid nitrogen for transport back to the lab and stored them in a -80 °C freezer. Protein extractions have been done and western blot analysis using CDPK antibodies is ongoing.

RESULTS AND CONCLUSIONS:
Complete data analysis on all samples is underway. However, several conclusions can be drawn. Significant effects were observed on seed development. C99R with no

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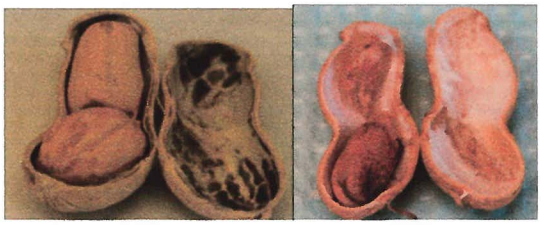
gypsum application had more hollow and unfilled pods compared to the gypsum treatment (Fig. 1), and to Georgia Green with no gypsum application. However, there was no reduction in the size of the pods. Calcium analysis revealed that pods contain more calcium than seeds, and it remains unclear whether this partitioning was altered under the calcium treatments. Further analysis is being conducted.



(A) (B)

Figure. 1: (A) mature pod and seed of C99R from the gypsum treatment, (B) mature pod and seed of C99R from the no added gypsum treatment.

Initial results of CDPK analysis have shown that CDPKs are expressed in all stages of seed development (stages 1-4). Also, CDPKs were detected in pods early in development. The pattern of expression of CDPKs in all stages of pods and seed under the calcium treatments is underway.

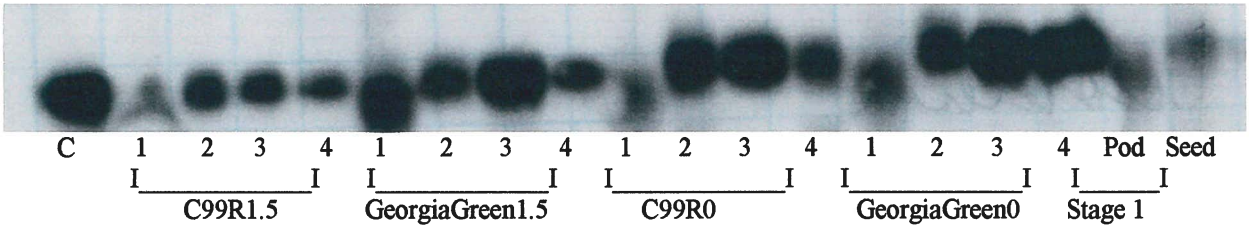


Figure 2. CDPK Western blot on developing peanut seeds and pod with (1.5) and without (0) calcium application.

EXPENDITURES: A Ph.D. candidate works on this project. Funds were also used for services, such as calcium analysis which was conducted by Waters Agricultural Laboratories, Inc. and supplies and chemicals used in the experiments including liquid nitrogen, extraction buffers, blot membranes, tubes, tips, membranes, etc.