

## Final Summary Report to the Virginia Peanut Growers

**Title: Petitioning Federal Agencies for Deregulated Status of Engineered Peanuts  
Project # 457621**

**P.I.: Elizabeth Grabau**

**Plot Location: Tidewater Agricultural Research and Extension Center**

### **Project Objectives:**

The objectives of the 2012 project were to:

1. Further increase Blight Blocker peanut seed in the field
2. Analyze potential risk of Blight Blocker peanuts to agriculture and the environment
3. Conduct first year field testing of High Folate peanuts
4. Determine seed folate content of harvested High Folate peanut lines

### **4<sup>th</sup> Quarter Results (October through December 2012 with Extension)**

#### **2012 Blight Blocker field plots**

To address **Objective #1**, Blight Blocker lines were increased in the field in 2012. In addition, to address **objective #2**, a plot of transgenic P39 Blight Blocker peanuts was planted in a larger field of non-transgenic Bailey peanuts. the frequency of gene flow between Blight Blocker and Bailey can determined by the number of resulting Bailey progeny that contain the OxOx gene. We expected frequencies to be low as was previously observed for Blight Blocker peanuts plants located adjacent to non-transgenic Perry cultivar (see frequencies below).

In this case, it was also hoped that natural outcrossing would occur. Even if at a low frequency, this would yield a Bailey-Blight Blocker progeny that could then be back crossed to Bailey to introduce the Ox Ox trait into the Bailey background.

Peanuts from the the Bailey plot were harvested and bulked. Individual progeny plants are being grown in the greenhouse and tested for the presence of the OxOx gene. To date, after examination of over 900 plants, only a single outcrossing event has been detected. The single plant is currently being grown in the greenhouse. In addition, more progeny plants are being analyzed for the presence of the OxOx gene.

This also confirms previous findings that risk of out-crossing in transgenic Blight Blocker peanuts is very low (1 in 900 = 0.11%).

## **DNA Sequencing of Blight Blocker Genome to investigate insertion events of Oxalate Oxidase Gene:**

In addition to our stated objectives, we also conducted sequencing on the P39 Blight Blocker line. The sequencing was performed by the SeqWright company and analyzed by the Virginia Tech Bioinformatics Institute. Unexpectedly, the results indicated 17 insertions of the transgene in P39 Blight Blocker peanut line. At this time, characterization of all 17 inserts at the molecular level is not possible. Once the Peanut Genome Project is completed, it should facilitate further characterization of the inserts. However, in the interim, we will proceed with further sequencing of additional lines to examine insertion of the Oxalate oxalate gene in other Blight Blocker lines, with hopes that we will have only a single or perhaps two insertions.

Additional peanut lines to be sequenced:

- N70 (derived from NC7)
- W171 (derived from Wilson)
- W73 (derived from Wilson)

DNA extractions were conducted to obtain sufficient DNA to submit for library construction and sequencing.

DNA has been examined for quality and quantified. A quote for sequencing has been obtained from the Virginia Bioinformatics Institute and DNA will be delivered in the near future.

## **First year field testing of folate peanuts (Objective #3)**

Presumptive high folate peanut lines were increased in the field in 2013. Folate lines tested are shown below along with the pod yield. Lines are color-coded to match the planting chart supplied in an earlier quarterly report. Preliminary folate analyses were conducted on peanut lines (see below).

**Perry Check            19.7 lb pods**

### **Folate Lines:**

<b>142-36-4-1-1B</b>	<b>2.9 lb pods</b>
<b>142-46-2-8B</b>	<b>8.1 lb pods</b>
<b>142-4-1-4B</b>	<b>8.1 lb pods</b>
<b>142-4-1-4-3B</b>	<b>4.4 lb pods</b>
<b>142-5-5-2B</b>	<b>10.2 lb pods</b>
<b>142-36-2-2B</b>	<b>12 lb pods</b>
<b>142-36-4-1B</b>	<b>13.2 lb pods</b>
<b>142-46-2-8B</b>	<b>8.1 lb pods</b>
<b>142-55-1-1-1</b>	<b>0.7 lb pods</b>
<b>142-55-1-1B</b>	<b>11.2 lb pods</b>

## Folate Analysis (Objective #4)

To analyze peanuts for folate content, seed samples were ground in a coffee mill.

- Peanut meal was shipped to Medallion labs for folate testing in two separate batches (no viable seeds shipped).
- Results were inconclusive (see below). Folate levels among samples of the same seed batch did not give consistent results.
- Consultation with Medallion revealed that the grinding process was probably the culprit because it was done at room temperature.
- Samples will be resubmitted and Medallion will conduct the grinding under liquid nitrogen conditions to avoid lipid pooling and uneven sampling.
- Shipping of seed will be conducted with APHIS approval.

Folate values for peanuts ranged from 87 to 569 ug per 100 gm weight. Results from the two different analyses are presented in graph form below.

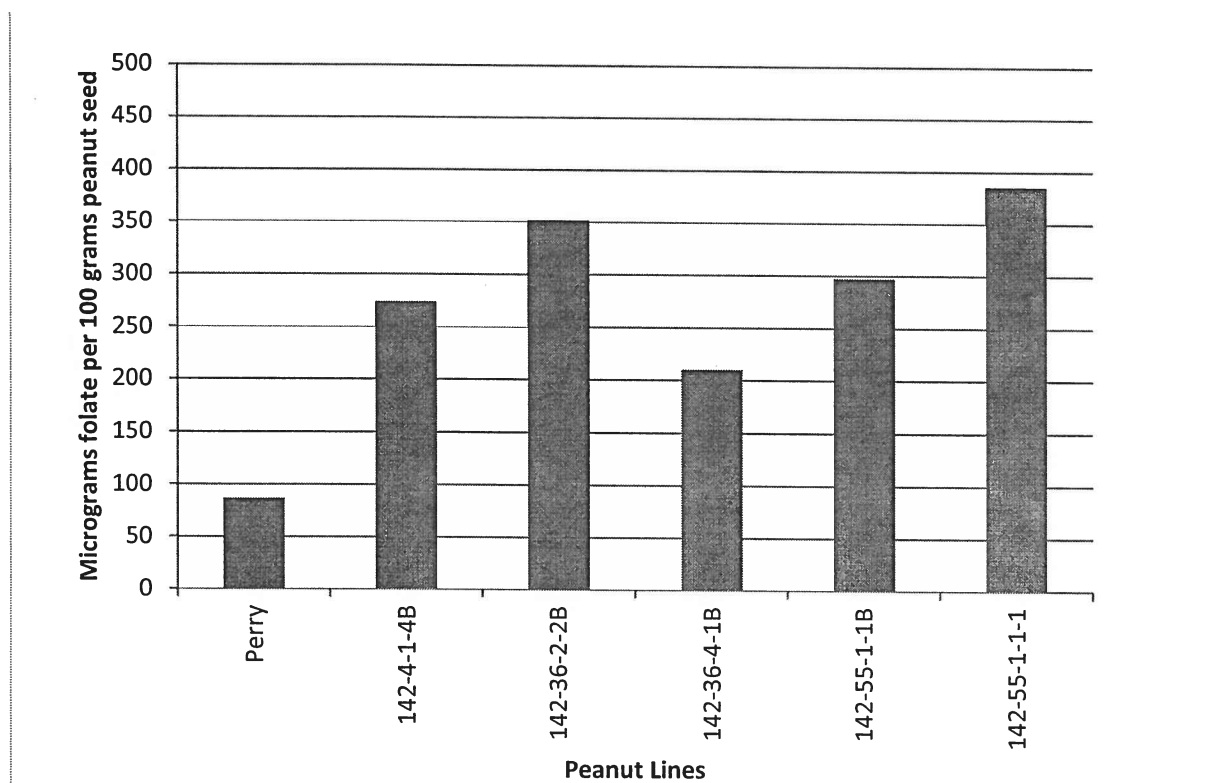


Figure 1. Micrograms of folate per 100 grams of peanut meal. Without duplicate samples, it appeared that all peanut lines were enhanced for folate. However, results were not consistent with a second set of analyses (see below).

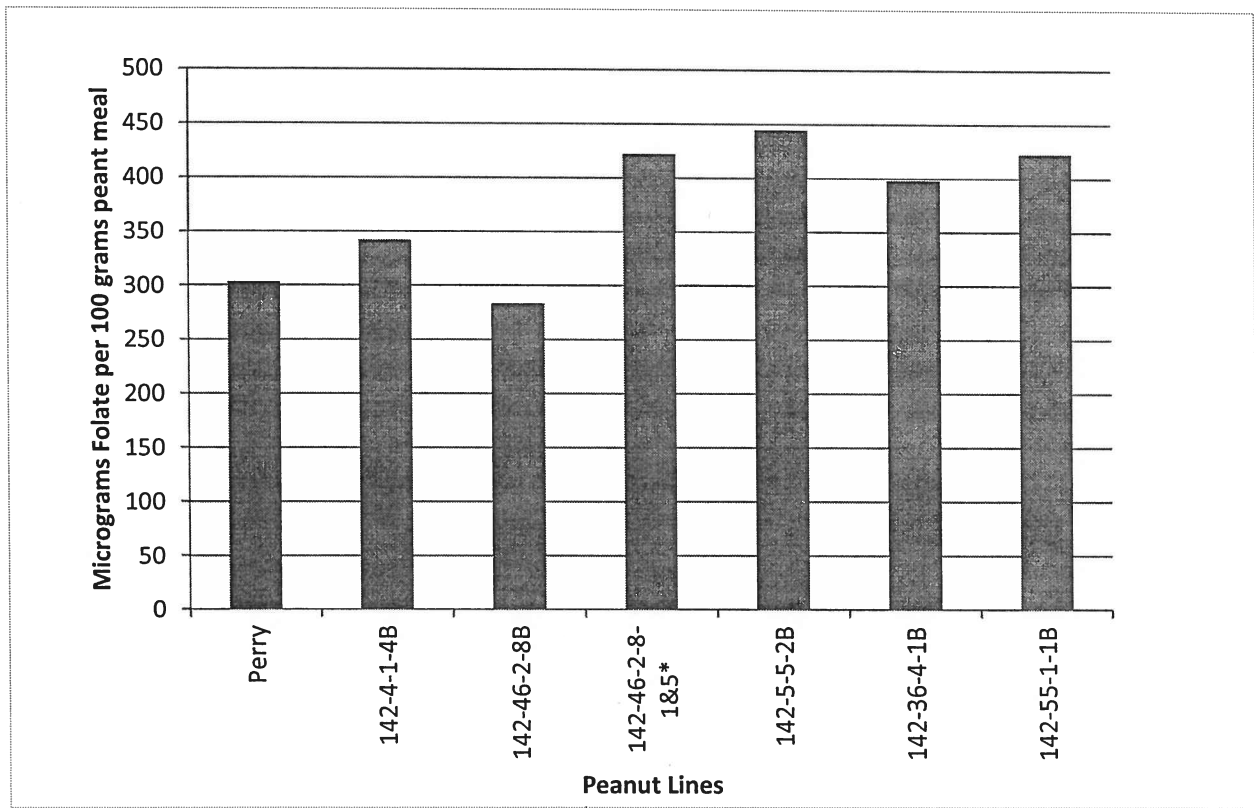


Figure 2. Micrograms of folate per 100 grams of peanut meal.

Results for control Perry meal was not equivalent to the first test. Upon consultation with the laboratory conducting the analyses, sample preparation is the likely culprit. We will retest all peanut lines using a different extraction procedure.