Final Project Report to the Virginia Peanut Growers Association
For January 1 – December 31, 2005

Grant title: Enhancing disease resistance in Virginia-type peanuts

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Project Goals for 2005:

The stated objectives of the 2005 project were:

1. To conduct the second year of field trials with expanded plot size for defining resistance to Sclerotinia blight and yield of transgenic lines with the oxalate oxidase gene
2. To produce additional transgenic lines of Virginia-type peanut with genes for glucanase, chitinase, and the nucleocapsid protein of TSWV.

Project Results for 2005:

We met 2005 project objectives of conducting a second year field test of transgenic peanut lines and generating new transgenic lines containing genes for chitinase enzyme (for additional fungal resistance) and/or the TSWV nucleocapsid gene (for resistance to tomato spotted wilt virus) as described below.

1. In 2005, 19 peanut lines previously recovered from tissue culture plus three non-transformed parent lines (NC 7, Wilson, Perry) were planted in replicated plots in the field. Plants were evaluated for gene expression and disease incidence at intervals during the growing season. Gene expression assays confirmed that 15 peanut lines expressed the barley oxalate oxidase resistance gene. Sclerotinia blight incidence was monitored on September 9, October 4, and October 27, 2005. A report entitled “Sclerotinia blight resistance in virginia-type peanuts transformed with the barley oxalate oxidase gene, 2005” was written and submitted for publication in Biological & Cultural Tests for Plant Disease Control. This report included a detailed comparison of disease and yield between parent and transgenic lines in the field trial. Little or no Sclerotinia blight was detected in transgenic lines expressing the oxalate oxidase gene compared to non-transformed cultivars. Yields for transgenic lines were greater than the non-transformed parent lines, with 10 of the transgenic lines yielding significantly better than the non-transformed parents. The yield increase of superior transgenic lines compared to the non-transformed parent ranged from 1042 to 1427 lb/A greater in NC-7, 435 to 792 lb/A greater in Perry, and 975 to 1124 lb/A greater in Wilson.

2. Peanut embryogenic tissue cultures were established from peanut cultivars in 2004 and used for bombardment with additional resistance genes in 2005. Peanut plants shown in the attached photographs were recovered from these cultures. The regenerated plants are being assayed for the presence of resistance genes.
Summary of Results:

- Wound inoculations of 4 wk-old plants of 15 transgenic lines with the barley oxalate oxidase gene in the greenhouse confirmed expression of resistance to Sclerotinia blight as seen in the field.
- All 15 T3 lines had reduced tissue damage on leaves exposed to oxalic acid.
- Eight of the 15 T3 lines inoculated with *S. minor* in the greenhouse had reduced lesion development over 7 days when compared to their non-transformed parent, and 10 of the T3 lines had reduced disease severity.
- Gene expression was confirmed in 11 of the 15 T3 lines showed gene expression was confirmed in 11 lines in the greenhouse.
- Disease appeared first in non-transformed cultivars and increased to high levels.
- Low to only a trace of disease was detected in lines with gene expression.
- Results confirmed heritability and functionality of gene in providing resistance.
- Ten of the transgenic lines showed superior disease resistance and yielded significantly better (435 to 1427 lb/A higher) than their non-transformed parents.

These following photos were taken in October 2005. The transgenic line is on the left and non-transformed parent is on the right for each pair. Plots were two, 25-ft rows.

NC 7 (transformed line and parent)

Perry (transformed line and parent)

Wilson (transformed line and parent)
Oxalate oxidase enzyme expression in transgenic lines planted in the field, 2005

(* significantly different from non-transformed parent)

Field evaluation of T3 transgenic lines for resistance to Sclerotinia blight, 2005
(AUDPC = area under the disease progress curve during the growing season)
Pod yield of transgenic lines (blue) and non-transformed parents in field trial, 2005.

Photos below are of seed and pods of non-transformed parent (top) and a transgenic line (below).
New transformed plants of Gregory (G) and Wilson (W) from the 2005 bombardment of tissue cultures with genes for improved disease resistance. These lines will be tested for gene expression and disease resistance in the future.

<table>
<thead>
<tr>
<th>Stage in regeneration</th>
<th>Number of transformants</th>
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<tbody>
<tr>
<td></td>
<td>Chitanase</td>
</tr>
<tr>
<td>In growth chamber</td>
<td></td>
</tr>
<tr>
<td>(4-6 plantlets each)</td>
<td>W: 19</td>
</tr>
<tr>
<td>Plants transferred to</td>
<td>W: 10</td>
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<tr>
<td>soil in the greenhouse</td>
<td></td>
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</tbody>
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Peanut plants regenerating in Magenta boxes

Plantlets transferred to soil