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Final
2006

**Enhancing Disease Resistance in Virginia-Type Peanuts**

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Peanut growers in Virginia have the highest production cost of most growers in the United States, because Virginia-type peanuts require higher seeding rates, supplemental calcium and intensive use of chemicals for disease control. Soil fumigant for control of Cylindrocladium black rot (CBR) costs growers about $30/A, and fungicide for control of Sclerotinia blight costs growers as much as $70 to $105/A. Sclerotinia blight does not occur in the southern U.S. (Georgia, Florida, and Alabama) where warmer temperatures have prevented establishment of the disease. CBR is also less damaging in the Southeast as a result of higher soil temperatures and lower susceptibility of many runner-type cultivars, which are grown throughout most of the region. As a result, yields of runner-type peanuts in the Southeast have continued to increase to levels that allow some farms to average above 4,000 lb/A, whereas grower yields in Virginia commonly average near 3,000 lb/A due to heavier losses of yield to disease.

**PROGRESS TO DATE:**

Our research has documented that *Sclerotinia minor*, the soilborne fungus that causes Sclerotinia blight, produces oxalic acid, a compound that predisposes plants to infection and disease. To combat the harmful effects of oxalic acid, Dr. Grabau isolated a single gene from barley that would allow plant cells to destroy oxalic acid and slow or prevent disease development. Over 300 modified plants were produced from Virginia-type cultivars Wilson, Perry and NC-7. After confirming the presence of the oxalate oxidase gene and its expression, plants with the gene and acceptable growth habits were selected for field trials.

Field trials in 2004 and 2005 evaluated the second and third generations of transgenic lines, respectively. Gene expression was confirmed in both generations growing in the field and greenhouse. In both years disease appeared first in the non-transformed parent cultivars and caused severe disease. Low to only trace levels of disease were detected in superior transgenic lines, which confirmed the heritability and functionality of the oxalate oxidase gene in providing resistance against Sclerotinia blight. In 2005, 14 transgenic lines expressing the oxalate oxidase gene had an average of 81% less disease than their non-transformed parents. All lines yielded equal to or better than their non-transformed parent with six lines yielding 436 to 1125 lb/A greater than their non-transformed parent and increased value by $123 to $252/A.

In 2006 we conducted the third year of field trials with expanded plot size for defining resistance to disease and yield. Four trials were designed to test for genetic uniformity and the acceptability of transgenic lines when released as commercial cultivars. Field trials also characterized disease resistance to Sclerotinia blight and other important diseases (early and late leaf spot, web blotch, southern stem rot, and CBR). Agronomic and quality traits of transgenic lines and their non-transformed parents were evaluated in cooperation with the PVQE program. Included were growth habit, yield, market characteristics, and quality (i.e. pod size, kernel grade and size, value/cwt, pod brightness, blanching efficiency, fatty acid profile, iodine value, calcium content).

All transformed lines in the first trial had reduced Sclerotinia blight with an average of 82.9 % less disease than their non-transformed parents in 2006 (Fig. 1). All lines yielded equal to or better than their non-transformed parent, while 10 lines yielded 479 to 2222 lb/A more with an increase in value of $90 to $422/A after grading (Fig. 2). Pod brightness, nutrient characteristics and fatty acid profiles are currently being evaluated on seed harvested from this trial.

In a second trial six transformed lines were compared to their non-transformed parents for susceptibility to other diseases such as tomato spotted wilt, leaf spot, web blotch, southern stem rot and Cylindrocladium black rot (CBR). Gene expression was confirmed in all six lines and incidence of Sclerotinia blight was an average of 83.8% less in the transformed lines as compared to non-transformed NC 7, Perry and Wilson. No significant differences in susceptibility were detected between the transformed lines and their non-transformed parents to tomato spotted wilt, early leaf spot, web blotch, and southern stem rot on October 4. Three lines of Wilson with the gene from barley did show increased defoliation over their non-transformed parent. All transformed lines had similar susceptibility to CBR, except for one which had significantly more CBR on 4 Oct. All lines yielded equal to or better than their non-transformed parents, except one transformed line of Perry.

Twenty-two single plant selections made in 2005 were hand planted in a third field trial to identify lines with homozygous gene expression and disease resistance. All 22 lines had plants with gene expression, reduced lesion size when leaf tissue was wound inoculated with oxalic acid and reduced Sclerotinia blight when compared to their non-transformed parent. Twelve lines had 100% of plants with gene expression, while the remaining lines ranged from 71% to 97.5% of plants with gene expression.

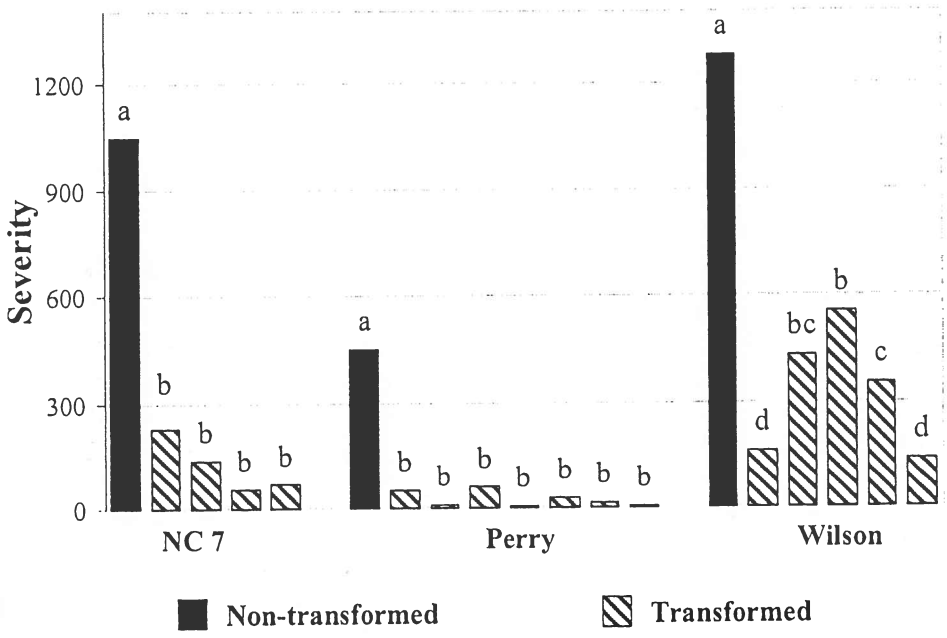
The fourth field trial was set up to evaluate the potential for the oxalate oxidase gene to outcross with non-transformed cultivars. Two transformed rows were planted with one non-transformed row between them and seven non-transformed rows on each side for a total of 17 rows per plot. Three replicates were planted for NC 7, Perry and Wilson. The peanuts were harvested at the end of the season and a sub-sample of seed collected from each non-transformed row will be planted in the greenhouse and evaluated for oxalate oxidase expression to determine potential for transfer of the oxalate oxidase gene to non-transformed cultivars.

Efforts to maintain regenerable embryogenic tissue cultures are ongoing using the cultivars Perry, Wilson, Gregory and NC 7. We have utilized embryogenic cultures for inserting additional genes to produce transgenic plants with improved disease resistance. As mentioned above, TSWV resistance is a major focus of our current experiments. In the past year we have stacked or pyramided the viral resistance genes in combination with the genes conferring resistance to fungal pathogens (oxalate oxidase, chitinases, and/or glucanases). We have introduced multiple gene combinations into the four major cultivars to regenerate additional plants for disease testing. Plantlets of Gregory and Wilson with combinations of chitinase, oxalate oxidase, and the TSWV nucleocapsid genes were regenerated in the Fralin Center at Virginia Tech. $T_1$ plants have been germinated and are currently growing in the greenhouse at the Tidewater AREC.

**POTENTIAL IMPACT:**

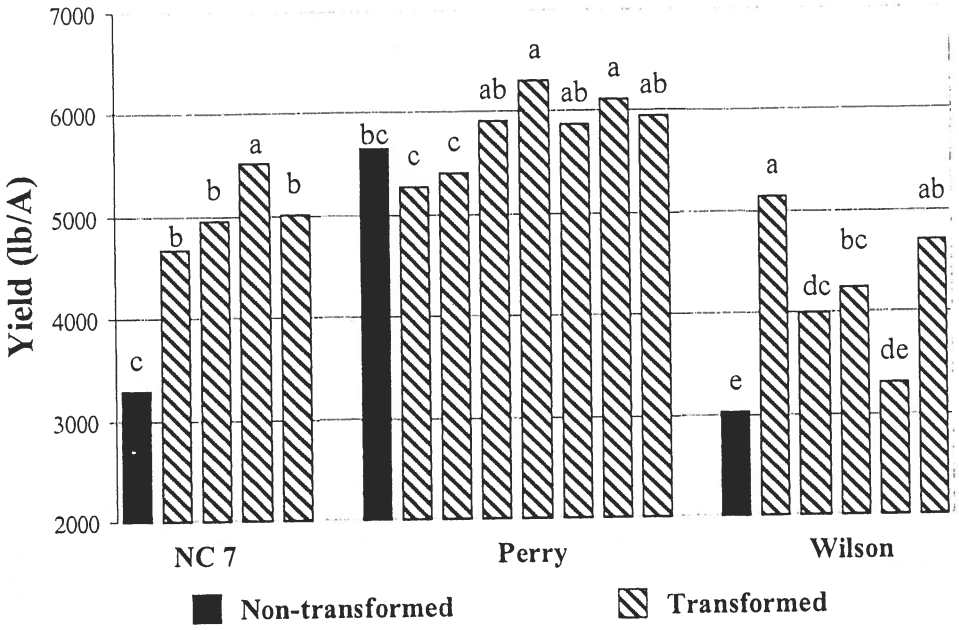
The expression of oxalate oxidase for enhancement of resistance to *S. minor* and other important diseases of peanut is expected to reduce yield losses to disease and the dependency on expensive fungicides for disease control. By reducing the cost of disease management and improving yield, this project offers promise for improving the profitability of growing Virginia-type peanut. Research conducted in 2006 is expected to expedite the development of disease-resistant cultivars with high yield, and essential market traits that are necessary for the Virginia peanut industry to be a more sustainable enterprise through the 21$^{st}$ century.

**Figure 1. Sclerotinia blight resistance in transgenic peanut lines**




**89% less disease in transformed lines than non-transformed parents.**

**Figure 2. Yield of transgenic Virginia-type peanut lines**




**479 to 2222 lb/A increase in yield.**
**$90 to $422 increase in transgenic lines vs. non-transformed parent.**