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National Peanut Board Research Project Report:
Blight Blocker Peanuts for Improved Resistance to Sclerotinia Blight

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The 2009 project focused on completion of documentation required to petition government agencies for deregulation and release of transgenic lines of peanut with an oxalate oxidase gene from barley. Field trials in 2009 were conducted at the Tidewater AREC in Suffolk with transformed lines and their non-transformed parents (Perry, Wilson and NC 7). Two trials compared incidence of Sclerotinia blight in parent cultivars and two advanced generations of transformed lines of each cultivar in plots with and without two applications of Omega 200 1 pt/A for control of Sclerotinia blight. Both trials were in fields with a history of Sclerotinia, and one of those fields also had a history of severe losses to *Cylindrocladium* black rot (CBR). Disease monitoring included incidence of early and late leaf spot, tomato spotted wilt, Southern stem rot, Sclerotinia blight, and CBR. Both fields were fumigated with Vapam 7.5 gal/A for control of CBR. Plant assays were done twice during the season to confirm the presence or absence of the oxalate oxidase gene in parent cultivars and transgenic lines. Disease incidence was monitored at two to three week intervals from July 1 until harvest. Yield was determined in both trials.

Sclerotinia blight trial: Sclerotinia blight was found first in non-transformed cultivars on August 6 in the field with a history of only Sclerotinia blight. All six of the transgenic lines for release were confirmed to express oxalate oxidase and exhibited high levels of resistance to Sclerotinia blight based on counts of disease incidence and area under the disease progress curve (AUDPC) throughout the growing season. AUDPC in the trial with only Sclerotinia blight problem was reduced 96% in transformed lines compared to their non-transformed parent cultivars, whereas Omega fungicide treatments reduced AUDPC in non-transformed parent cultivars by 55%. All six of the transgenic lines at harvest had significantly lower incidence of Sclerotinia blight and AUDPC either with or without Omega fungicide. Yields in plots without Omega fungicide were significantly higher for transformed cultivars when compared to their non-transformed parent. Incidence of other diseases included low levels of tomato spotted wilt and CBR, and counts were not significantly different across parent cultivars and transgenic lines.

Sclerotinia blight and CBR trial: The first observations of Sclerotinia blight in this trial were in non-transformed cultivars on August 6. As in the trial above, gene expression was confirmed in all transgenic lines, and all exhibited high levels of resistance to Sclerotinia blight. AUDPC for Sclerotinia blight in this trial was reduced an average of 93% by transgenic lines, whereas Omega fungicide reduced AUDPC in the non-transformed parent cultivars by an average of 54%. Incidence of tomato spotted wilt was low, but CBR incidence in the NC 7 and Wilson parent cultivars and their transformed lines was equally high and not significantly different. As expected, CBR incidence in partially-resistant Perry and its transformed lines was lower than levels in non-transformed and transformed NC 7 and Wilson. Yields of non-transformed NC 7 and Wilson were equally low in their transformed lines due to heavy CBR pressure. Non-transformed Perry yielded higher than NC7 or Wilson and yields of transformed Perry lines also showed an increase (not significant) over that of non-transformed Perry.

Comparisons of food chemistry: A comparative analyses of kernel chemistry for non-transformed cultivars and transformed lines included factors such as iodine value (shelf life), fatty acids, oleic and linoleic ratio (O/L), polyunsaturated/saturated ratio (P/S), mineral content

(Ca, K, P, S, Mg), blanching efficiency, and food label data. Comparisons of differences between parent cultivars and their corresponding transgenic lines were not significant or lacked biological significance. In addition, multivariate data were analyzed by a canonical discriminant analysis. The first three canonical functions were significant at $P=0.0001$ and accounted for more than 92% of cultivar variation. O/L, P/S, iodine value, percentages of fancy pods and extra large kernels were the most differentiating traits. These traits separated the cultivars into three, distinct clustered groups of NC 7, Wilson, and Perry with their corresponding transgenic lines. The three clusters were significantly different based on the pairwise Mahalanobis distance ($P=0.01$), but there were no significant differences between transformed lines and their corresponding parent. These findings provided evidence that parent cultivars and their corresponding transgenic lines are similar in kernel chemistry. This finding is in agreement with results in previous years concerning grade characteristics, fatty acids, minerals, food value of peanut hay, kernel brightness, and blanching. In addition to these data analyses, an independent lab was contracted to perform kernel analyses for food labels on parent cultivars and their corresponding transgenic lines in 2009. The results showed the percentages of nutrients were similar in non-transformed cultivars and their transgenic lines with the oxalate oxidase gene.

Summary: Overall, data indicated that transformation with oxalate oxidase was specific for Sclerotinia blight and not other diseases or pests. Transformation of a peanut cultivar with oxalate oxidase did not result in significant changes in kernel or hay chemistry, and did not increase the risk of aflatoxin in kernels. An additional field study is in progress to determine the frequency and distance of outcrossing in 2009 between transgenic and non-transgenic lines. Previous trials have indicated that the greatest potential for outcrossing and gene flow between non-transgenic and transgenic lines occurred within 4.5 m from a transgenic pollen source. Average rates of gene transfer were 0.47, 0.42, and 0.13% in NC 7, Perry and Wilson, respectively. Beyond 4.5 m, gene flow occurred in a random fashion. The maximum distance for detection of an outcrossing event was 17.1 m in field trials conducted in 2007 and 2008. The field trial in 2009 was designed to measure outcrossing up to distances of 150 m. Field observations indicated that bumble bees and honey bees were most likely responsible for cross pollination of peanut flowers and gene flow. A petition for deregulation and release of superior transgenic lines with 5 years of data from laboratory, greenhouse and field studies from 2005 through 2009 is near completion for submission to APHIS, EPA, and FDA in 2010.