Reid Shalow

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A Report to the
North Carolina Peanut Growers' Association, Inc.
P.O. Box 8
Nashville, NC 27856-0008

on Research Entitled

Establishment and Operation of a Peanut Transformation Facility

Leaders:

Arthur K. Weissinger
H. Thomas Stalker
Principal Investigators
Department of Crop Science
North Carolina State University

We are currently carrying out three projects in the Peanut Transformation Facility. In the first, we are working to develop transgenic peanut lines with improved resistance against *Aspergillus flavus*, in an attempt to reduce the likelihood of aflatoxin contamination. These lines are transformed with a gene, Mod 1, derived from corn. Mod 1 encodes a ribosome inactivating protein (RIP)that is known to retard the growth and development of *Aspergillus spp. in vitro*. The gene is driven by a potato ubiquitin promoter (Ubi 3) that is expected to result in the expression of the protein in all tissues of the plant.

Transgenic peanut lines have been produced in peanut cultivars 'Georgia Green', a runner type, and in 'NCV 11', a Virginia type. Transgenic plants have been shown to express the RIP protein during all stages of plant development. The protein accumulates in very significant quantities in the outer cell layers of the seed, thereby accumulating the defensive protein where growth of the *Aspergillus* fungus is most likely to occur.

We have demonstrated that seeds from peanut plants that make this protein are highly resistant to a toxigenic strain of *Aspergillus flavus*. These lines have all now been advanced to the F_3 generation.

We are also working with Drs. Tom Isleib, peanut breeder in the Department of Crop Science, NCSU, and Dr. Corley Holbrook, peanut breeder with USDA-ARS at Tifton, GA, to transfer the Mod 1

gene from the original transgenic lines to other peanut varieties that express natural resistance against aflatoxin contamination. We anticipate that some of the progeny of these crosses will exhibit both transgenic and natural resistance against Aspergillus and aflatoxin contamination, and that this resistance will be stronger and more durable than either source alone.

We have also tested Mod 1 transgenics against *Sclerotinia minor* and *Sclerotium rolfsii* using an assay developed by Dr. Barbara Shew, Plant Pathology, NCSU. At least four lines have exhibited statistically significant levels of resistance in these assays. We are currently repeating these tests. We are also in the process of testing these same lines against early and late leafspot diseases and Cylindrocladium Black rot.

In the second project, a gene encoding the coat protein from tomato spotted wilt virus (TSWV) has been introduced into peanut varieties NC-V 11. Numerous transgenic lines carrying this gene were recovered and have been grown to maturity in the greenhouse. Nineteen lines (i.e., independent transgenic events) have been shown to have complete resistance against the Hawaii L strain of TSWV, which is the isolate from which the gene conferring resistance was originally isolated.

With Dr. Isleib, we are carrying out crosses to transfer the TSWV resistance transgene from transgenic lines to TSWV susceptible peanut varieties, such as Perry, increasing their utility in areas where the virus is prevalent.

A gene encoding an artificial storage protein (ASP 1) that can be used to improve the amino acid balance in peanut protein has been introduced into peanut cv. 'Georgia Green'. Expression of ASP 1 could improve the amino acid balance of peanut, and could potentially lead to the development of an improved peanut that could be marketed as a novel value-added product.

A total of 78 transgenic plants derived from 60 independent transformation events have now been recovered. These lines are now being advanced to the R2, and we are in the process of producing seeds to be used in large-scale testing of these lines. At present, we are working to develop Federal funding to support the further characterization of these lines, and to study how modification of the protein composition of these peanuts will affect their overall performance. Of special interest is any alteration of yield or pest resistance normally present in the recipient lines.

Finally, we have now completed the move of the Plant Transformation Laboratory to a much more modern and better-equipped facility on Centennial Campus. This will provide significantly more space for peanut transformation work, and will put us into closer proximity with colleagues involved in development of plant transformation technologies. Also, a new

Senior Researcher has been hired by the College of Agriculture and Life Sciences to work on transformation projects. We anticipate working with this person to develop a more efficient, *Agrobacterium*-based transformation protocol for peanut.

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IMPACT STATEMENT

The projects that are currently being carried out in the Peanut Transformation Facility address the need of North Carolina peanut growers to have peanut varieties that are more productive and less expensive to produce. They also address the need for new, value-added peanut products that can be used to develop new markets for American peanuts.