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A Report to the
National Peanut Board

on Project **NC~~4~~ 19**

Development of Transgenic Peanut Varieties with Improved Agronomic Performance and Enhanced Protein Quality

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We are currently carrying out two major 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*. In the current reporting period, we also have proven that these lines exhibit statistically significant reduction in aflatoxin B1 accumulation when inoculated with toxigenic *A. flavus*. This observation is critically important because it proves that aflatoxin contamination can be managed by engineering peanuts to express a fungicidal protein.

We have also tested Mod 1 transgenics against *Sclerotinia minor* and *Sclerotium rolfsii*, both of which cause serious leaf blights that result in economic losses to growers. Further, we have obtained preliminary results suggesting that this same transgene is also effective in reducing infection by leafspot. We are also in the process of testing these same lines against early and late leafspot diseases and *Cylindrocladium* Black rot.

Importantly, if field tests confirm our experimental observations, the use of this transgene may result in a significant reduction in the need for fungicide applications used to control these

foliar diseases. This could represent significant savings in cost of production to growers, and aid in reducing losses due to aflatoxin contamination.

We are also working with to transfer the Mod 1 transgene 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.

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. Importantly, this strain is an extremely virulent pathogen, and we have found that plants that can resist infection by this strain are also highly resistant to all other isolates of the virus that we have been able to test.

We have begun the process of acquiring appropriate permits from USDA-APHIS to conduct field grow-outs of all of these materials in summer 2007. WE anticipate that these field tests will provide more reliable data about the utility of these materials for combating diseases than we can achieve in greenhouse tests. Further, because pod yields are significantly greater in the field, this grow-out will allow us to produce the large amounts of seed needed to conduct subsequent field trials over a number of locations in North Carolina, Virginia and Georgia.

Although we have had few resources that could be committed to the project, we are also working to develop transgenic peanuts with "consumer traits", specifically, improved nutritional content. 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. We have produced numerous transgenic plant lines that express the protein. However, at present we do not have the resources needed to conduct biochemical tests to determine the extent to whicch expression of the gene alters protein composition. We anticipate that it may be possible to obtain additional funding to complete this project through either international aid agencies, or through philanthropic foundations, such as the Rockefeller Foundation.

We have also prepared and will submit a proposal to USDA-NRI for support to develop transgenic peanut lines with increased levels of Co-Q_{10}, a critical nutrient found in peanuts. Deficiency of this nutrient is associated with chronically poor diets, aging and certain disease states, such as some forms of cancer. Further, it has been shown that supplementation with Co-Q_{10} can have significant benefits to patients with certain chronic heart diseases. Finally, plants in which this compound has been elevated show increased resistance to stressors such as disease, drought and high temperatures.

We hypothesize that the level of this critical nutrient can be increased by the manipulation of a single metabolic step in the biosynthetic pathway by which it is made in plants. We will produce transgenic peanuts carrying this alteration, and determine its effect on Co-Q_{10} accumulation.

This project offers three major benefits to the peanut community. First, plants with enhanced resistance to stress will undoubtedly become increasingly important as elements of advanced agricultural systems with which to confront current both current environmental

challenges, and the extreme environmental conditions predicted to be associated with changes in global climates. Secondly, enhancement of Co-Q10 levels would improve the nutritional quality of peanuts, which could then be marketed as identity-preserved products for direct consumption and for the producing high-value processed foods. Finally, peanuts exprssing large amounts of this compound could be extracted using conventional peanut oil extraction technologies to produce a cost-effective dietary supplement. Currently such supplements are produced by yeast or bacterial fermentation, and their cost is very high. Reducing this cost would simultaneously make such supplements available to a broader spectrum of consumers, and expand market opportunities for peanut producers and processors.