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Molecular Approaches Toward Producing TSWV-Resistant Peanut

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Objectives:

1. To isolate peanut defense-response genes, particularly those involved with increased resistance to tomato spotted wilt virus (Results see below).
2. Field test, at multiple locations, one line of peanut that is transgenic for a TSWV gene, along with evaluating several other transgenic lines that show promise.

In general, the use of insecticides alone to control thrips vectors has been an ineffective means of suppressing tomato spotted wilt virus (TSWV). One exception to these findings was the discovery that in-furrow application of the systemic insecticide phorate suppressed epidemics of the disease. Phorate (Thimet 20G) has demonstrated consistent, low-level suppression of TSWV. The mechanism of TSWV suppression by phorate is unknown, but the level of thrips control obtained with phorate is not greater than that obtained with other insecticides. Therefore, it is unlikely that TSWV suppression is due to a decrease in vector population. Observations in the field revealed that phorate can be phytotoxic, causing marginal necrotic lesions on peanut leaves. We hypothesize that phorate may induce a defense response in the peanut plant that allows it to better resist TSWV. To understand the components of a phorate-induced response in peanut that may condition acquired resistance to tomato spotted wilt, we have used differential display of mRNA to identify gene products that are regulated by phorate treatment. Thirty-one out of a total of fifty-three clones were down regulated and the remaining twenty-two were up regulated. All fifty-three clones were sequenced and analyzed. The sequences were subjected to data base comparison using BLAST and showed strong similarities with known sequences whose products are involved in a variety of cellular functions. (This work won the 2002 Bailey Award at the APRES meeting held in NC.)

In an effort to achieve TSWV protection, we are utilizing an approach of pathogen-mediated resistance in which we genetically engineered peanut with transformation vectors that contained the DNA sequence of the nucleocapsid protein gene of TSWV. Transgenic peanut progenies, produced from transgenic lines that were recovered after microprojectile bombardment and showed production of both mRNA and nucleocapsid protein in the plants, were subjected to natural infection of the virus under field conditions in the growing seasons of 1999 and 2000 in Tifton, GA and in three locations (Tifton, GA; Marianna, FL; Headland, AL) in 2001. Significantly lower infection rates were observed on the transgenic progeny in comparison to the non-transgenic control

plants. DNA and ELISA analysis of the transgenic progeny showed that the nucleocapsid protein gene was stably integrated into the peanut genome at a single locus and segregated in a 3:1 ratio, implying that those transgenic plants could be readily used in a traditional breeding program. This result demonstrated the possibility of enhancing host resistance through introducing a virus gene into peanut plants. (This work was nominated for a Bailey Award at the 2002 APRES meeting).

The transgenic TSWV-resistant line was resistant to TSWV and is being used in crosses with: 1) breeding lines from Corley Holbrook that contain resistance genes from *A. hypogaea*, and 2) other transgenic lines that contain fragments of the viral nucleocapsid protein gene.