## Progress toward development of root-knot nematode resistant transgenic tomatoes for control of *Meloidogyne* spp.

Conkling, M.A., Opperman, C. H., Bird, D. McK, Cheah, K., Skantar, A.M., Acedo, G. N., Saravitz, D. M., and D. T. Kaplan.

<sup>1</sup>Depts. of Plant Pathology and Genetics, N.C. State University, Raleigh, NC and Subtropical Plant Pathology Research Unit, USDA-ARS, Orlando, FL.

Root-knot nematodes are obligate plant parasites that induce development of an elaborate feeding site during root infection. Feeding site formation results from a complex interaction between the pathogen and the host plant in which the nematode alters patterns of plant gene expression within the "giant cells" destined to become the feeding site. Our goal is to interfere with development of the feeding site via either the specific expression of toxin genes within the giant cells, or alteration of plant gene expression patterns in the developing giant cells. The nematode's inability to establish a proper feeding site would lead to death of the pathogen due to starvation. The tobacco root-specific gene, TobRB7 encodes a water channel protein that is expressed in the developing vascular tissue in uninfected roots and in giant cells during infection by root-knot nematodes. Analysis of the TobRB7 promoter has revealed cis-acting elements responsible for root-specific and nematode inducible expression of the gene.

Our approach to develop stable nematode-resistant tomato lines involves developing transgenic plants with antisense constructs of nematode-responsive plant genes specifically invoked in giant cells. These genes are up-regulated in giant cells and may be involved in either giant cell development or cell maintenance. A nematode attempting to feed on cells carrying a nematode-inducible promoter fused to an antisense construct of one or more of these genes may not develop normally. Antisense constructs would prevent genes required for giant cell development and maintenance from functioning properly. The goal of this project is to limit nematode development by curtailing giant cell development in tomato and thereby reduce crop loss due to root-knot nematodes which are currently controlled using methyl bromide soil fumigation.

The TobRB7 promoter fused to a  $\beta$ -glucuronidase reporter gene (GUS) and transformed into tomato. Transgenic tomato plants were infected with *Meloidogyne incognita* and *M. arenaria*. Following infection, the roots were stained histochemically for GUS expression. As predicted, GUS was expressed exclusively in giant cells. We have also identified and cloned the TobRB7 homologue from tomato which will also be used for tomato transformation.

Tomato transformation efficiency is also variety dependent. However, we have optimized tomato transformation which has enabled us to regenerate most, if not all, commercial varieties. Our transformation now ranges from 70 - 100% whereas, standard protocols may only yield 2-16% for some commercial tomato varieties.