Expression of Anti-Nematode Proteins in Transgenic Soybean to Control Soybean Cyst Nematode

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Progress report (covers period of 1/1/2007-12/31/2007)

Objective: Confirmation of soybean cyst nematode (SCN) control in stable transgenic soybean plants expressing ANPs.

Progress made toward objectives:

Generation of constructs:

We initiated a program to identify proteins with nematicidal activities and to express their cognate genes in the roots of transgenic soybean plants. The patent and open literature was searched for proteins and peptides that displayed antimicrobial and/or insecticidal properties. A total of 438 sequences were identified and grouped by bioinformatics into 52 families and 60 singlets. Sequences were selected according to the following criteria: source of organism; activity of the peptide (potency and spectrum); size of the peptide; and structure. Thirty sequences were further chosen, cloned into a plant ubiquitin expression vector and tested for plant toxicity using *A. rhizogenes*-mediated leaf infiltrations. Those not exhibiting plant toxicity were expressed in soybean roots using the composite plant protocol. Composite plants (wild-type shoots with transgenic roots) expressing the proteins/peptides were then challenged with SCN. Analysis of three replicated trials identified seven protein/peptides which significantly reduced the level of SCN infestation. Ubiquitin expression vectors for each peptide were cloned into a soybean plant transformation vector containing a GUS scorable marker and a BAR selectable marker.

Soybean transformations:

All soybean ubiquitin peptide/GUS/BAR expression vectors were electroporated into the disarmed *A. rhizogenes* strain, 18r12v (developed in the Taylor laboratory), and used for production of stable transgenic soybean plants. Soybean *cv*. Hutcheson was chosen for plant transformation due to it susceptibility to soybean cyst and root-knot nematodes. Soybean transformation was optimized using empty vector CGT 6400. Currently, all constructs are being transformed into soybean. Transformation efficiencies for soybean *cv*. Hutcheson is estimated to be around 2%; however, this number is subject to change since experiments are still ongoing. In total we have produced 35 transgenic plants and expect more to be produced from ongoing experiments (Table 1). Once transgenic plants have been taken to seed, T1 plants will be examined for protein expression and selectable marker segregation. Once enough seed is produced, transgenic soybean plants will be tested for resistance to SCN.

Table 1: Transgenic soybean plant (cv. Hutcheson) production:

CGT 6400	Empty vector	6 Plants + Experiments underway
CGT 6512	Paradaxin	3 Plants + Experiments underway
CGT 6513	Ta ITX2	3 Plants + Experiments underway
CGT 6514	Empty carrier vector	2 Plants + Experiments underway
CGT 6535	Plectoxin	Experiments underway
CGT 6536	Aptotoxin	7 Plants + Experiments underway
CGT 6537	Defensin A	3 Plants + Experiments underway
CGT 6538	DTX 9.2	6 Plants + Experiments underway
CGT 6539	Diptericin	5 Plants + Experiments underway

Tomato transformations:
As an alternative to soybean transformation we also produced transgenic tomato lines that express the ANP constructs. Tomato was chosen because it can serve as an alternate host for SCN. Dr. Terry Niblack (University of Illinois, Urbana-Champaign) maintains several inbred lines of SCN that have been selected for their ability to grow on soybean and tomato. The three strongest ubiquitin/peptide constructs identified from the composite plants system and two control constructs were used for plant transformation. Transgenic tomato (*cv.* Moneymaker) plants were produced using standard protocols developed at the DDPSC Tissue Culture Facility. Seed from these tomato plants have been collected and await segregation analysis, protein expression analysis and screening for SCN resistance (Table 2).

Table 2: Transgenic tomato plant (cv. Moneymaker) production:

CGT 1448	Empty vector	25 Plants
CGT 1777	Empty carrier vector	25 Plants
CGT 1716	Paradaxin	14 Plants
CGT 1717	Ta ITX2	13 Plants
CGT 1729	DTX 9.2	10 Plants

Development of Western detection protocols for identifying ubiquitin/peptide expressers:
Because no antibodies are available at this time for the nematode-lethal peptides, we will monitor the protein expression using the HA-tag that is fused to the N-terminus of the ubiquitin protein. By monitoring expression of the fused ubiquitin, we will be able to predict which lines are likely to have the highest levels of expression. Using protein extracts from tobacco plants homozygous for the ubiquitin/peptide constructs, we have developed a Western protocol to identify the best expressing transgenic lines. Once enough material is developed for soybean, we will use the same protocol to identify the best expressers.

Future Plans

We will finish the plant transformations and begin the testing of soybean and tomato plants for segregation analysis, protein expression and screening for resistance to SCN.