

Mutagenesis of the Root-Knot Nematode Resistance Gene Mi-1.2

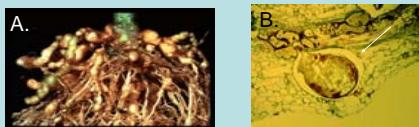


Introduction

Root knot nematodes (*Meloidogyne* spp.) are a major agricultural pest that cause millions of dollars in damage to crops annually. These microscopic worms infect plant roots causing them to produce galls and reduce plant growth. However, not all plants are susceptible to nematodes. Some plants contain a resistance gene called Mi-1.2 that give them pathogen resistance. The goal of the project is to figure out how this gene works. I used site-directed mutagenesis to study how changes in the nucleotide sequence of the MI-1.2 gene affect its ability to confer resistance to nematodes.

1. Nematodes in Tomato Roots

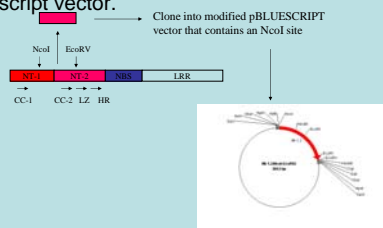
Nematodes infect root cells and cause plants to undergo physiological changes to produce galls. A. Galls caused by *M. incognita* on tomato roots. B. Adult female feeding in the root (white arrow).



M. javanica infected-tomato roots

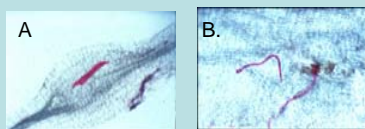
2. M-1.2

The Mi-1.2 resistance gene has four domains. The Nt-1 and Nt-2 are effector domains, NBS is a nucleotide binding domain and LRR is the leucine-rich recognition domain. We chose the region between Nco1 and EcoRV (NT-2) restriction sites for site-directed mutagenesis. We cut this region out and put it into the bluescript vector.



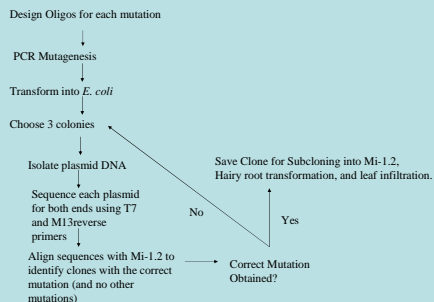
3. Comparing Resistant and Susceptible Plants

A: Large nematodes in susceptible plants without Mi-1.2 grow and develop in the roots.
B: Stunted nematodes in resistant plants die in the roots when Mi-1.2 is present.



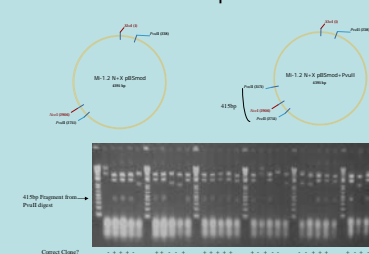
4. Overview of Subcloning

This is a schematic of the procedure we used to mutate specific nucleotides in the Mi-1.2 gene. We first created the mutant genes using polymerase chain reaction (PCR), then transformed these genes into the bacteria *E. coli*. We then analyzed the sequence of the genes to determine whether we had a positive mutant with a single nucleotide change.



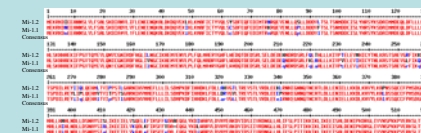
6. Screening Clones Using Added PvuII Site

We ran a gel of the purified plasmids to see if the added PvuII site was there. This confirmed that our mutated NT-2 insert was present in some clones (+).



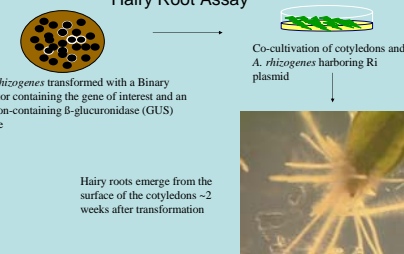
7. DNA Sequencing

Once we knew our insert was present, we sequenced it to confirm that the single-base mutation was correct.



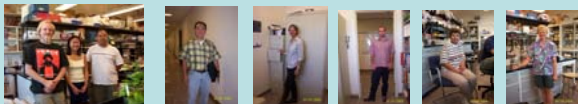
8. Conclusions and Future Directions

We have made a number of constructs with single-nucleotide changes that will be tested for resistance to nematodes. We plan to transform plants using the hairy root assay (below). If one of the transformed plants turns out to be susceptible to nematodes, then we can identify which nucleotide change blocks resistance.

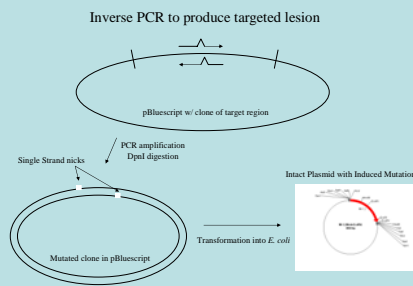


Acknowledgements

I would like to thank my mentor, Adam Telleen, for teaching me many wonderful things about Mi-1.2 and science in general; Qingli Liu, Chin-Feng Hwang, and especially Steve Lower for helping me on my poster; Wacława Pudło, Luma AlBanna, Ann Liu and everyone else in the lab for support; Valerie Williamson for taking me into her lab; and Barbara Soots for accepting me into the program.



5. Site Directed Mutagenesis



Mutations in the NT-2 fragment were made using Polymerase Chain Reaction (PCR), a technique for amplifying DNA. We designed primers with the mutations we needed and ran a PCR reaction of the Mi-1.2 region in bluescript. The plasmids with inserts, which were mutated, were then transformed into *E. coli* by heat shock.