Identification and Characterization of C-Type Lectin genes from the Reniform Nematode, *Rotylenchulus reniformis*

By

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Outline

Introduction to C-type Lectins (CTLs)
Identification of CTLs in Reniform Nematode
Characterization of CTLs in Reniform Nematode
Conclusions & Future Directions
Why study C-type lectins in reniform nematode?

- High-frequency EST from cDNA library of sedentary female life stage (Wubben et al., 2010)
- Major component of innate immune response system
- Other functions - cell adhesion, host-recognition

What is innate immunity?

- First line of defense in an organism
- Non-specific immune response
- Evolutionarily older defense strategy
Lectins:

- Proteins with at least one non-catalytic domain that bind reversibly to a specific carbohydrate

![Diagram of a cell membrane with a domain](image)

C-type lectins:

- Lectins which require calcium for their activity
- Single or multiple carbohydrate recognition domains
Reniform Nematode (Rotylenchulus reniformis)

- Semi-endoparasitic on many plant species
- Annual cotton yield losses of over $130 million
- Parasitic stage - Adult female
- Syncytium - feeding site in plant roots
Life cycle of *Rotylenchulus reniformis*

- **Egg**: Day 2-3
- **Second Stage Juvenile (J2)**: Day 10-11
- **Third Stage Juvenile (J3)**: Day 18-21
- **Fourth Stage Juvenile (J4)**: Day 24-25

- **Molt-I**: Egg mass on root
- **Molt-II**: Female feeding on root
- **Molt-III**: Host Infestation
- **Molt-IV**: Adult Female

- **Adult Male**
Structure of CTL gene in reniform nematode

5’ UTR → Exon 1 (D) → Exon 2 (D) → CTL Domain → Exon 3 → 3’ UTR

Translation

Signal Peptide → Calcium Binding Site
PCR Amplification of CTL Domain

- Six life stages under study
- Conserved domain of 155 amino acids

- Full length gene amplified only in sedentary female
- 5’ and 3’ RACE of CTL domain from other life stages
  - First Choice RLM-RACE Kit (Life Technologies)
Full-length Gene Amplification

PCR Amplification of Full-length CTL

- Conserved domain region
- Variability beyond the domain
### CTLs expressed in reniform nematode

- Three CTLs common to all life stages
- Eight other CTLs in specific life stages.

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<th>Rr CTL</th>
<th>Size (bp)</th>
<th>Egg</th>
<th>J2</th>
<th>J3</th>
<th>J3J4</th>
<th>Adult Vermiform</th>
<th>Sedentary Female</th>
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Southern Hybridization

To find the copy number of CTL genes in the genome
Real-Time Expression Analysis of CTL Domain

To study relative-fold expression across life stages
**in situ Hybridization**

To locate the site of expression in sedentary female

**Anti-Sense** DIG-labeled CTL Domain Probe Hybridization

**Sense** DIG-labeled CTL Domain Probe Hybridization
Phylogenetic Analysis - CTL Domains
Conclusions

• CTLs have a possible role in establishing parasitism
• Sedentary female is the most sensitive stage to target
• Each stage-specific CTL may have a specific role

Future Directions

• Study expression under microbe-induced conditions
• Evaluate role in infection through gene-silencing
• Develop nematode-resistant transgenic cotton plants
Questions?