Identification of New Esophageal Gland Effector Candidates from Adult Females of the Root-Knot Nematode

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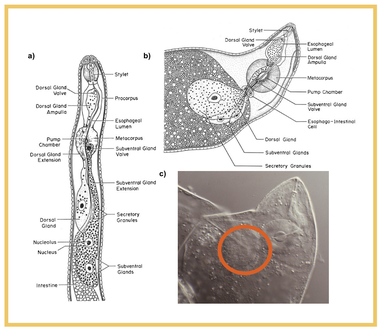
INTRODUCTION

The root-knot nematode (RKN) *Meloidogyne incognita* represents one of the most economically important species of plant-parasitic nematodes. RKN has a world-wide distribution and the ability to infect virtually any cultivated crop species. Through a hollow, protrusible stylet, these nematodes secrete effectors to manipulate host cell structures and function for their own benefit. These effectors are produced by highly specialized secretory esophageal gland cells, one dorsal and two subventral, whose roles differ throughout the nematode life cycle (Figure 1a and b).



Elucidating the transcriptomic profile of these gland cells has proven to be a promising approach for identifying new effectors involved in nematode parasitism during its motile and sessile phases. Prior gland isolation studies have focused on juvenile stages of the nematode. In this study, we developed a protocol to enrich the highly active dorsal glands from *M. incognita* adult females for RNA extraction and identification of novel effector candidates.

Figure 1. Illustrations of the anterior regions of sedentary endoparasitic nematodes showing the esophageal gland secretory cells. (a) A migratory, infective second-stage juvenile with the two active subventral esophageal gland cells. **(b)** A swollen female from within infected roots with reduced subventral gland cells and an enlarged active dorsal esophageal gland cell. Illustrations reproduced from Hussey et al. (1994). **(c)** Photomicrograph of a female head displaying an active dorsal gland.

METHODS

Dorsal gland collection and enrichment

Manual cutting

+

Sonication / Vortexing

+

Cell strainer Filtration

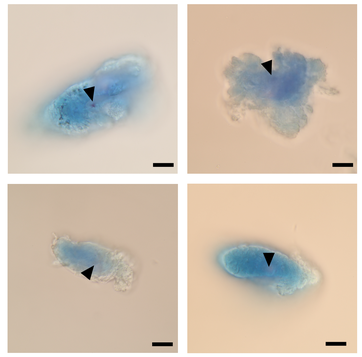


Figure 2. Collected glands following enrichment protocol. Glands were stained with HistoGene staining solution. Arrowheads indicate nucleus location. Scale bar is 20 µm.

RNA isolation / Library construction
Sample groups:
Pre-Parasitic Second-Stage Juveniles 2 (ppJ2)
Female Heads (FH)
Dorsal Glands (DG)

RNA-seq analysis
Mapping to *Meloidogyne incognita* v3 reference genome
Differential gene expression (DESeq2 and edgeR)

RESULTS

- 37,160 unique genes (of the 43,718 coding genes in the genome) were identified across the three experimental samples

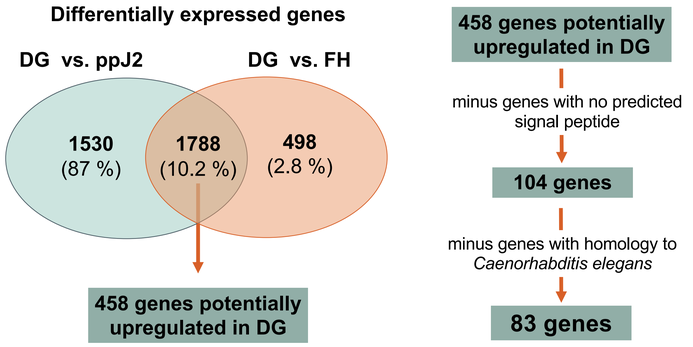


Figure 3. Diagram showing sample filtering of candidate *Meloidogyne* effector genes. Pair-wise comparison of dorsal glands with ppJ2 and female heads identified 17,089 and 2,286 differentially expressed genes (DEG), respectively. Out of the two sets of DEG, 1,788 genes were common in both comparisons, with 458 genes upregulated in dorsal glands samples and, thus, of interest to this study. Out of the 458 genes upregulated in dorsal gland samples, 104 contained a predicted signal peptide and lacked transmembrane domains. Of these genes, 83 showed no homology to proteins in the free-living nematode *C. elegans*.

- 59 genes were previously not known to be expressed in dorsal glands

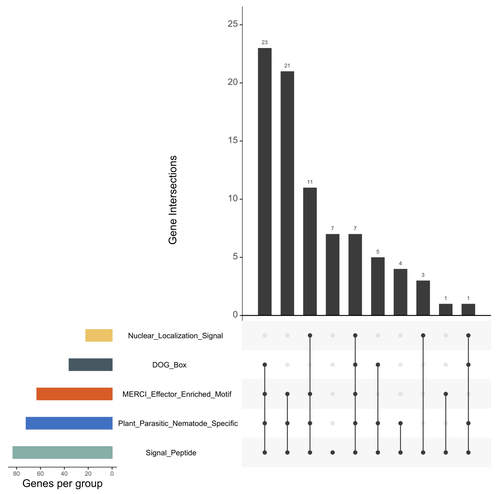


Figure 4. Upset plot of candidate *Meloidogyne* effector genes. An UpSet plot was generated from additional analyses of the 83 dorsal gland effector candidates, which showed that 36 genes had a DOG box promoter motif (Da Rocha et al., 2021), 22 contained a nuclear localization signal (NLS), and 63 contained a predicted MERCI effector motif (Grynberg, et al., 2020) within their encoded protein sequences.

***In situ* hybridization**

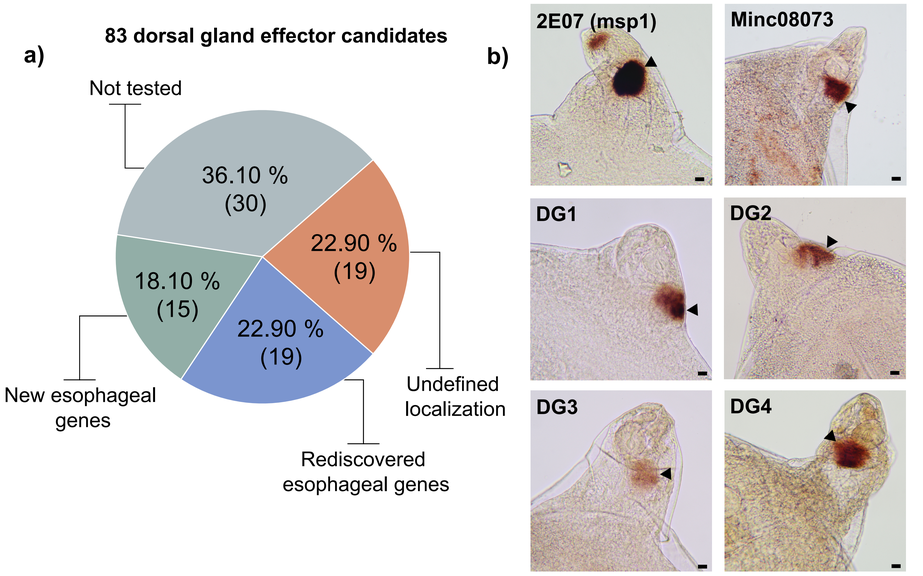


Figure 5. *In situ* hybridization of *M. incognita* adult females. (a) Summary of dorsal gland effector candidates localization **(b)** Digoxigenin-labeled antisense DNA probes to transcripts expressed within the dorsal gland (DG) cell. Sense cDNA was used as negative control and showed no signs of staining. Arrowheads indicate DG cell. Scale bar is 10 µm.

CONCLUSION

Taken together, we have identified novel candidate *Meloidogyne* effector genes that may have important roles during later stages of parasitism. Ongoing studies will further elucidate the role these candidate effector genes play in *the M. incognita*-host interactions.

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