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Identification of Egg Development Genes in *Meloidogyne incognita* as Potential Targets for Nematode Control

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ABSTRACT: Silencing genes associated with egg development and fertility in *Meloidogyne incognita* using RNA interference (RNAi) offers a potential avenue for effectively managing this destructive crop pest. To identify potential targets, we selected the gene *vit-2* based on its functional role in the model nematode *Caenorhabditis elegans*. The *vit-2* gene, part of the vitellogenin gene family, is essential for yolk production, which supports post-embryonic larval development and fertility. In silico analysis of *Meloidogyne incognita* using BLASTn and tBLASTx search tools led to the identification of the key target gene, Minc3s00286g09435. Primers for the identified gene were designed using the PrimerQuest tool from IDT/the Primer3 Plus software. The presence of the gene in *Meloidogyne incognita* was confirmed through polymerase chain reaction (PCR) followed by gel electrophoresis. A partial sequence of 716 bp was obtained for the *vit-2* gene. With the confirmed presence of the gene, it can be targeted through RNA interference (RNAi) to investigate its functional role in *Meloidogyne incognita*. Confirming the gene's function could enable RNAi-based control of *Meloidogyne incognita*, enhancing sustainable pest management and crop resilience.

Keywords: *Meloidogyne incognita*, RNA interference (RNAi), *vit-2* gene, egg development, fertility, PCR, gel electrophoresis, sustainable pest management.

INTRODUCTION

Nematodes likely evolved around 400 million years ago, predating the Cambrian explosion (Poinar, 1983). These unsegmented, bilaterally symmetrical, triploblastic animals have a pseudocoelomic body cavity. Key damaging genera include *Meloidogyne, Rotylenchulus, Heterodera, Radopholus, Pratylenchus, Globodera, Aphelenchoides*, and *Tylenchulus*. Root-knot nematodes (RKNs, *Meloidogyne* spp.) are the most economically important plant-parasitic nematodes, causing around 5% of crop losses globally (Taylor and Sasser 1978), with greater losses in developing countries. Among 101 species identified worldwide (Seid *et al.,* 2015), 16 are found in India. The dominant species—*M. incognita, M. javanica, M. arenaria*, and *M. hapla*—are responsible for 90% of damage (Castagnone-Sereno, 2002; Moens *et al*., 2009). In India, *M. graminicola* incurs the highest economic loss in rice, approximately Rs. 23,272.32 million annually (Kumar *et al.,* 2020), while *M. incognita* mainly affects vegetables and horticultural crops.

The genome of *Caenorhabditis elegans* was sequenced previously; however, numerous other nematode genomes have since been sequenced (The C. elegans Sequencing Consortium, 1998). Discovered in *Caenorhabditis elegans* by Andrew Fire and Craig C. Mello—Nobel laureates in 2006—RNAi functions through a sequence-specific mechanism. The genome of *Meloidogyne incognita* was sequenced by Abad *et al*. (2008). Genomic research can reveal critical stages in the life cycle of obligate parasites that may be unique and serve as specific targets for anti-nematode therapies. RNA interference (RNAi) is an emerging tool for developing novel management strategies against plant-parasitic nematodes (PPNs) through gene silencing. To identify housekeeping genes as potential RNAi targets, we will utilize functional genomic data from the *C. elegans* genome and conduct genomic comparisons using bioinformatics techniques.

Nematode eggs, ellipsoidal and resilient, have three layers: vitelline, chitinous, and lipid. The vitelline layer, from the oolemma, supports vitellogenesis and post-embryonic development (Bird and Bird 2012; Perez and Lehner 2019). In *Caenorhabditis elegans*, six vitellogenin genes are highly expressed, with vitellogenin-6 protecting against oxidative stress, while vitellogenins 1-5 do not (Sornda *et al*., 2019). Vitellogenins, crucial for yolk protein synthesis, are highly expressed in the intestine of *Caenorhabditis elegans*, where they support egg development and fertility. The *C. elegans* vit-5 gene shares 96% similarity with vit-3 and vit-4, and 67% with vit-2. Vit-6 has a 50% similarity to vit-2, while vit-1, similar to vit-2 at 82%, is a pseudogene due to truncation (Perez

et al., 2017). Egg development in plant-parasitic nematodes (PPNs) may represent a critical weak point in their life cycle that can be investigated in detail. Although these mechanisms are likely conserved across nematode species, the specific processes may vary. The application of established in vitro and in vivo experimental methodologies to assess the effects of RNAi knockdown on nematodes would facilitate investigations into their egg development and multiplication, as well as the functional analysis of targeted genes. Should RNAi-mediated knockdown demonstrate efficacy, in planta approaches for developing resistance to root-knot nematodes (RKNs) could be explored. In their review, suggest that the identification and manipulation of stress-responsive genes in crops can enhance resilience against nematodes, potentially leading to improved crop management strategies in the face of climate change.

Research indicates that transgenic plants designed to silence housekeeping and other essential genes significantly reduce nematode survival rates.

MATERIAL AND METHODS

Identification and maintenance of *Meloidogyne incognita.* The single egg mass inoculation technique was used to culture *Meloidogyne incognita* on brinjal (*Solanum melongena).* Egg masses were collected from infected roots, and females were stained with acid fuchsin-lactophenol for species identification based on perineal patterns. Species-specific primers (Adams *et al.,* 2007) confirmed the population.Hatched juveniles (J2) from confirmed egg masses were inoculated onto susceptible brinjal plants (cv. Pusa Purple Long) in pots in a glasshouse, Division of Nematology IARI, New Delhi. After 2-3 months, egg masses were collected from infected roots and juveniles hatched using the modified Baermann method for further experiments.

Identification of key target genes through in silico analysis in *Meloidogyne incognita.* The study aimed to identify a gene playing a functional role in the egg development of M. incognita. Egg developmental genes involved in nematode intestine formation and yolk production in *Caenorhabditis elegans* were identified to study *Meloidogyne incognita*. Spliced coding sequences of *C. elegans* genes were retrieved from WormBase Parasite and aligned against *M. incognita* protein-coding transcripts, downloaded from both WormBase Parasite and Meloidogyne Genomic Resources, INRA(http://www6.inra.fr/meloidogyne_incognita). Sequences with high similarity at both protein and nucleotide levels were identified. These sequences were further confirmed using BLASTX and BLASTN against the *M. incognita* genome. Primers were then designed for amplification and further analysis.

Designing of primers for identified genes. After in silico analysis, a 716 bp fragment of the *Mi-vit 2* gene was analyzed using INTERPRO-SCAN to identify functional domains and checked in the dsCheck database for potential off-targets. Primers were designed using the Primer Quest tool of IDT or Primer 3Plus (http://eu.idtdna.com/primerquest/home/index). Table 1 provides the primer sequences**.**

Table 1 : Details of primer used in an experiment.

Primers used in PCR amplification of *vit-2* gene from cDNA of *M. incognita*		
Gene	**Gene specific primer (5'-3')**	**Amplicon length**
vit-2	*vit-2* 1F-ACAGGCTCGCTGTGCTCAA *vit-2* 1R-TAGTAGCCATTGCATCAACAAG	716 bp

RESULT AND DISCUSSION

***In silico* quest for the gene of interest:** A genome-wide search for the developmental gene *vit-2* based on the literature search and studies on the model nematode *C. elegans*. The gene sequence was retrieved using bioinformatics tools from *C. elegans.*Then the corresponding ortholog in *M. incognita* was retrieved from its genome.The resulting sequence was confirmed through BLAST search in the general database NCBI, nematode-specific database Worm Base Parasite, and *Meloidygyne-specific* database INRA. For the query and subject for sequence alignment were used *C. elegans* gene sequence and *M. incognita* sequence using NCBI-BLAST server respectively. From all the databases the selected sequences were used for further bioinformatic analysis as higher query coverage, lesser E value, etc. for BLASTn analysis, all the matched character of the sequence was used to gene identity in other nematodes. We identified the gene of interest in *M. incognita* using *in silico* analysis.

Table 2: Gene *vit-2* identified by bioinformatics analysis in *M. incognitain silico.*

Gene name	***Vit-2***
BLAST type	TBLASTX
Database location	FXSY01000286.1 7550 to 8152 (+)
Genomic location	FXSY01000286.1 7550 to 8152 (+)
Query location	Query_1 307 to 909 (+)
Alignment score	1082
E-value	0.0
Alignment length	201
Percentage identity	99.5
Gene length	5855
M. incognita transcript name	**Minc3s00286g09435**

Table 3: Function and expression site of interest gene

Gene	**Tissue/organ**	**Function**
Vit-2	Intestine	Enable lipid transporter activity and nutrient reservoir activity

In this present study, we have explored a development gene vitellogenin-2 (*vit-2*). The *vit-2* gene belongs to the vitellogenin gene family which is six in number *viz.,vit-1*, *Vit-2*, *vit-3*, *vit-4*, *vit-5*, and *vit-6* with yolk production and supporting post-embryonic development as their primary function (Perez *et al*., 2019). Among these, the *vit-2* gene has some distinct role in nematode

development other than the primary role of the vitellogenin gene family. Since its expression was reported in the abdomen and its proposed involvement in the *daf-16* pathway by Goszczynski *et al*. (2016) in the model nematode persuadedus to target this gene. The *vit-2* gene sequence has been retrieved from the *wormbase* database and the orthology search against *M. incognita* genome resulted in the gene sequence with Minc3s00286g09435.

CONCLUSIONS

In conclusion, this study highlights the distinct role of the vitellogenin-2 (*vit-2*) gene in nematode development beyond its traditional function in yolk production. The identification of an orthologous gene in *Meloidogyne incognita* (Minc3s00286g09435) suggests a conserved role for *vit-2*, potentially linked to the *daf-16* pathway. These findings point to *vit-2* as a promising target for novel nematode control strategies.

FUTURE SCOPE

Further research into the *vit-2* gene's role in nematode developmental pathways offers exciting potential for nematode management. Key areas of future exploration include uncovering its non-reproductive functions, particularly in stress response and metabolism, and investigating its interaction with pathways like *DAF-16/IIS*. Functional studies in parasitic nematodes, such as *Meloidogyne incognita*, could reveal conserved mechanisms, leading to cross-species control strategies. Techniques like RNAi and CRISPR-Cas9 may target *vit-2* to disrupt nematode development. This research could lead to novel biocontrol agents, agrochemicals, or crop varieties with enhanced resistance, offering sustainable solutions for nematode management.

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