SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)

SRI-VIPRA

Project Report of 2024: SVP-2434

"Study On Plant Parasitic Nematode-Responsive Defence Genes"

IQAC
Sri Venkateswara College
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SRIVIPRA PROJECT 2024

Title: Study on Plant Parasitic Nematode-Responsive Defence Genes

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List of students under the SRIVIPRA Project

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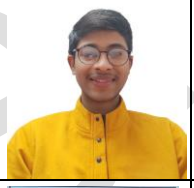
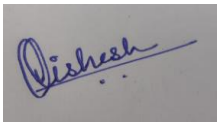
3		Vishesh Pandey	1122151	B.Sc. (prog) life sciences	
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Signature of Mentor: 

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2434 titled "Study on Plant Parasitic Nematode-responsive defence genes**"**.The participants have carried out the research project work under my guidance and supervision from 1st July, 2024 to 30thSeptember2024. The work carried out is original and carried out in an online/offline/hybrid mode.

M.Prajna

Signature of Mentor

Acknowledgments

We would like to begin by expressing our sincere gratitude to **Dr. Muthabathula Prajna** for her unwavering support, guidance, and expertise, which were fundamental to the success of this research on **"Plant Parasitic Nematode-responsive Defense Genes."** Her insights into plant pathology and molecular biology not only helped shape the direction of this research but also deepened our understanding of the genetic and metabolic pathways involved in plant-nematode interactions. Her critical contributions were essentially helped us to explore potential biotechnological applications for crop security.

We are deeply thankful to Sri Venkateswara College for providing us with the incredible opportunity to be part of the Sri Vipra research initiative. The platform, resources, and academic environment fostered by the institution were crucial in enabling us to explore new ideas and achieve our research goals. We would also like to extend our gratitude to the Sri Vipra team for their continuous support and encouragement throughout this project.

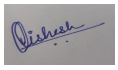
This project has been a true team effort, and we owe its success to the dedication and hard work of all students involved in this project. Their collaboration and insights have driven this research forward, making this journey an enriching and rewarding experience. Additionally, we express our appreciation to the lab staff for their consistent support and technical assistance, which were invaluable throughout the research process.

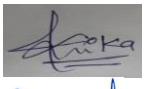
Finally, we are profoundly grateful to God for blessing us with the strength, wisdom, and perseverance needed to undertake this research. His guidance has been a constant source of inspiration, giving us the resilience to overcome obstacles and remain committed to our work.

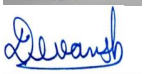
Together, with the combined efforts of our mentor, team, and the blessings of God, we have been able to accomplish what we set out to do. Thank you all for your contributions.

Abhay Panchal 

Shambhavi 

Vishesh Pandey 

Ishika Kapoor 

Devansh Dixit 

Anushka Singh 

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1.Introduction

It was the early 20th century when in the quiet corridors of agricultural research institutions, where the first hints of a problem were observed. Farmers, grappling with declining crop yields despite diligent care, were puzzled. Their fields, once abundant, now showed signs of stunted growth, yellowed leaves, and premature wilting. The usual suspects—disease, pests, or poor soil quality—seemed to offer no clear answers.It was in the early 1900s that scientists like Charles Thorne and Frederick H. Brunnemeyer began to connect the dots. Thorne, a pioneering plant pathologist, was among the first to identify that certain invisible pest were wreaking havoc below the soil. His meticulous research led to the discovery of root-knot nematodes, small but devastating parasites that infiltrated the roots of plants, disrupting their nutrient uptake (Hussey, R. S., & Barker, K. R.,1973).In the early days researchers would collect soil samples, painstakingly separate and identify nematodes, and then observe their effects on various plants. It was a laborious process fraught with trial and error, but each discovery added a new piece to the puzzle. The nematodes' ability to form galls on plant roots, impeding water and nutrient absorption, was particularly alarming (Brunnemeyer, F. H., 1943).

Nematode is a widely recognized group of a distinct phylum Nematoda or Nemata under superphylum Ecdysozoa (all moulting animal group). Early members of phylum Nematoda are thought to have evolved in marine habitats during the Cambrian period (600–550 million years ago). Nematodes can inhabit in marine, freshwater and terrestrial environments. They are the most abundant (approximately 0.5–10 million) multicellular animals on earth. Mostly their size is small (plant parasites and free livings, 0.25 mm to 12 mm; animal parasites 5 cm to 10 m) and they have a colorless body; thereby, they are inconspicuous organisms. Among the plant parasitic nematodes (PPNs), the most economically important ones are root knot(*Meloidogyne*), cyst (*Heterodera and Globodera*), seed gall *(Anguinatritici*), reniform(*Rotylenchulusreniformis*), citrus (*Tylenchulussemipenetrans*), burrowing *(Radopholussimilis)* stem and bulb (*Ditylenchus spp*.), foliar (*Aphelenchoides spp*.), lesion (*Pratylenchus spp*.) and pinewood nematodes (*Bursaphelenchusxylophilus*). Nematode infestation accounts for about 14 % global loss amounting to ~$100 billion dollars annually (Chitwood, 2003). In fact, PPNs constitute a small fraction (~15 %) of the described nematodes (~30,000 species), the majority being non-parasitic and free-living organisms that play essential roles in terrestrial and sediment food webs. Free-living nematodes constitute about 40 % of the described species, which mostly feed on bacteria (bacterivores), fungi (fungivores), algae, protozoan and diatom (unicellular eukaryote feeders) and other nematodes (predator), while animal parasites (or pathogens) of vertebrate and invertebrate (carnivores) and plant parasites (herbivores) share 44 % and 15 %, respectively, of the described species (Khan MR et.al. 2015).

Nematode displays conserved and simple body plan. Generally, the nematodes (Greek words *nema* (thread) and oides (form)) are pseudocoelomates (absence of mesodermal lining) and wormlike similar to unsegmented animals popularly known as roundworm (circular in cross section), eelworm (eel-like animal), nemas, threadworm (thread-like), etc. and generally lack external appendages. They are typically vermiform (with tapered ends), thread-like and fusiform (spindle shaped) and covered with a usually translucent, flexible, acellular cuticle secreted by an underlying cellular hypodermis. Most nematodes are sexually dimorphic (dioecious). Although they are generally oviparous, some are viviparous or ovoviviparous. The tail (post-anal portion) varies in shape from broadly rounded to filiform or intermediate forms, and it may differ

between the sexes and even developmental stages. In transverse section, they are vermiform and circular. They are triploblastic (comprise of three germ layers: ectoderm, mesoderm and endoderm) and pseudo coelomic and unsegmented (segmentation does not go below the hypodermal layer) animals and have bilateral (in sagittal section. They do not possess circular body muscles and their movement is accomplished by contraction and relaxation of longitudinal muscles. Their body cuticle is highly flexible and the body shape is maintained through pressure from body fluid which is analogous to hydrostatic skeleton (Khan MR et al. 2015).

Nematodes have complete digestive system (alimentary system), which consists of the pharynx (so-called esophagus) which has a lumen (triradiate in cross section) and the intestine which is a simple tube that is usually of single cell thickness on the peritoneal side and internally lined with microvilli. They lack the circulatory and respiratory systems. The nervous system mainly consists of circumcenter nerve rings. In female, the reproductive organs are in the middle to posterior of the nematode. Nematode species often have both male and female and often reproduce asexually by parthenogenesis. The reproductive organs are sometimes used as attributes for identification because the number of ovaries and the position of the vulva in the female nematode's body are easily seen under the light microscope. Male nematodes can be easily identified by the presence of spicules. Spicules are copulatory structures that are used during mating to guide the sperm into the vagina of the female nematode (Fig. 1) (O'Brien et al.1991).

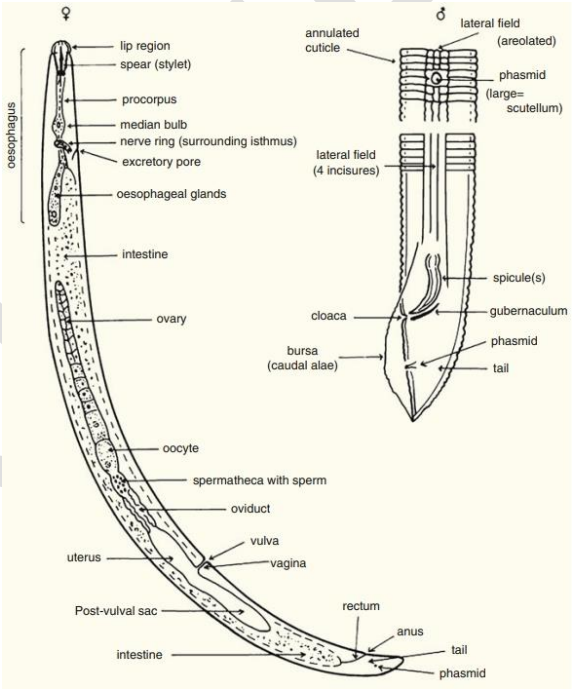


Fig.1Morphology of*Tylenchorhynchus*(From O'Brien and Stirling, 1991)

As the decades progressed, technological advancements began to shed new light on these cryptic creatures. The advent of electron microscopy in the 1960s provided an unprecedented glimpse into the nematode's anatomy and behavior, revealing their complex life cycles and interactions with plant roots in startling detail. Researchers like Dr. David J. Chitwood utilized these new tools to delve deeper, uncovering the nematodes' sophisticated mechanisms for manipulating plant tissues to their advantage. [Chitwood DJ (2003)]. The 1970s and 1980s saw a surge in interdisciplinary approaches. Plant geneticists and molecular biologists joined the fray, aiming to

unravel the genetic interactions between nematodes and their host plants. One breakthrough came with the development of resistant plant varieties. By understanding the nematodes' attack strategies, scientists were able to engineer crops that could thwart their efforts, either by producing chemicals that nematodes found toxic or by altering their root structures to resist infection. They discovered the mechanism of nematode infection in plants. Here's the mechanism described(Fig. 2).

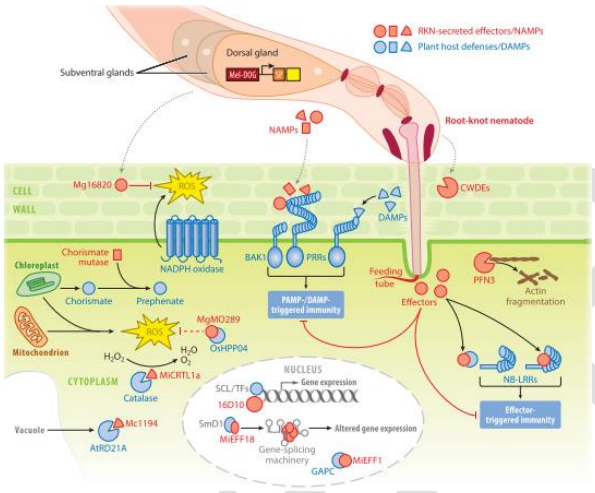


Fig.2: Advances in root-knot nematode (RKN)–plant interactions. RKNs possess a stylet that mechanically penetrates host-plant cells for feeding and effector secretion. Effectors are expressed in the esophageal glands, where they are secreted into the apoplast or directly into the cytoplasm. The cis-regulatory motif, Mel-DOG, is enriched in effectors expressed within the dorsal gland cell of Meloidogyne incognita. Apoplastic CWDEs are secreted to degrade and breach the plant cell walls. However, the host can perceive cell wall fragments, as well as NAMPs, that are released during infection and mount defense responses. In turn, RKNs secrete an arsenal of effectors to overcome plant defenses. The apoplastic effector Mg16820 interferes with PAMP-triggered immunity (PTI)-mediated production of reactive oxygen species (ROS). Additionally, RKNs balance cytoplasmic ROS abundance during infection by modulating ROS scavenging (dashed red line), as demonstrated with the effectors MgO289 and MiCRTL1a. Many effectors manipulate different plant immunity components to suppress defenses. The RKN effector Mc1194 targets the plant defense protease RD21A (Responsive to Desiccation 21) as a means to overcome host defenses. Chorismite mutases have also been shown to suppress defense responses. Effectors are also involved in giant cell formation and maintenance to acquire nutrients. PFN3 disrupts actin polymer formation during early giant cell formation. The interaction between host GAPCs and effector MiEFF1 in giant cells is also required for parasitism. Furthermore, the RKN effectors MiEFF18 and 16D10 hijack the host gene expression and the gene-splicing machinery, respectively, to modulate gene expression within giant cells. More studies are required to identify effectors that aid the nematode in evading components of PTI and effector-triggered immunity (ETI) as well as those recognized by host resistance genes. Abbreviations: CWDEs, cell wall–degrading enzymes; DAMPs, damage-associated molecular patterns; GAPCs, glyceraldehyde-3-phosphate dehydrogenases; NAMPs, nematode-associated molecular patterns; NB-LRRs, nucleotide binding site–leucine-rich repeats; PAMPs, pathogen-associated molecular patterns; PRRs, pathogen recognition receptors; SCL, SCARECROW-LIKE; SP, signal peptide sequence; TFs, transcription factor.

SOURCE- https://doi.org/10.1146/annurev-phyto-021621- 120943

With advancements in bioinformatics and Next Generation Sequencing, our understanding of the defensive genes involved in nematode infections and their interactions has greatly expanded. Thanks to developments in genomics and computational biology, we can now create improved plant hybrids that are more resistant to nematodes, ultimately leading to increased crop yields.Today, the field of nematology is a vibrant and dynamic area of research, supported by a global network of scientists who continue to push the boundaries of knowledge.

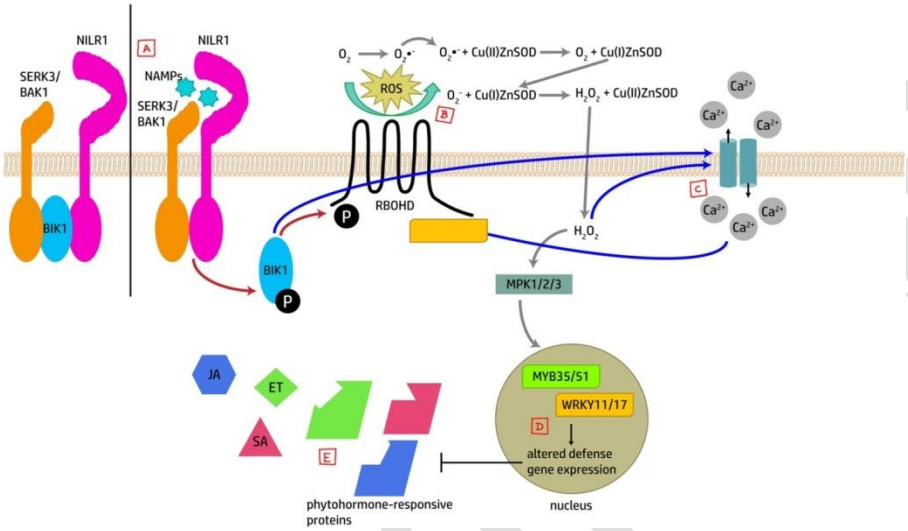


Fig.3Model of a PTI response pathway and nematode effector target point. NAMP recognition by PRRs (A), ROS production (B), calcium signaling (C), defense gene expression (D), phytohormone signaling (E) (Kelly Goode& Melissa G. Mitchum, 2022).

SOURCE-**https://doi.org/10.1111/ppl.13680**

In this project, we aim to investigate the defensive genes involved in nematode resistance in coffee and sugarcane plants, focusing on their associated mechanisms, utilizing bioinformatics. Our methodology includes several key steps. Initially, with the identification of genes known to confer nematode resistance across various plant species. Subsequently, extraction of the FASTA sequences which were subsequently subjected to pairwise sequence alignment using tools such as tblastn and comparing them against the genomes of coffee and sugarcane. The results of these alignments were analyzed to elucidate the presence and functional relevance of the defensive genes in conferring nematode resistance using bioinformaticstools such as DAVID, PDP, UniProt and MOTIF SEARCH.

2.METHODOLOGY

2.1SELECTION OF PLANT FOR ANALYSIS

Two plants, Sugarcane and Coffee were selected for the study, due to the following reasons:

2.1.1. SUGARCANE-*Saccharum officinarum*

1. **Economic Importance**: Sugarcane is a major global crop, vital for the production of sugar, ethanol, and other by-products. Its economic significance drives the need for effective pest and disease management, including nematodes, which can cause substantial yield losses.
2. **Nematode Susceptibility**: Sugarcane is highly susceptible to various nematode species, including root-knot nematodes (*Meloidogyne spp.*), cyst nematodes (*Heterodera spp.*), and others. These nematodes can severely impact root health, nutrient uptake, and overall plant growth, making it a pertinent subject for studying nematode resistance mechanisms.
3. **Complex Root System**: The complex root system of sugarcane, which includes both fibrous and adventitious roots, provides a diverse environment for nematodes to interact with. Studying how nematodes affect such a root system can offer insights into both general and specific resistance mechanisms.
4. **Genetic Diversity**: Sugarcane has a broad genetic base, including both commercial cultivars and wild relatives. This diversity is valuable for research into nematode resistance, as it allows for the identification of resistant varieties and the study of genetic traits associated with resistance.
5. **Biotechnological Potential**: Advances in genomics and biotechnology, including the availability of sugarcane genomic resources, facilitate the investigation of nematode resistance. Sugarcane's genome has been sequenced, and genomic tools are increasingly available, supporting research into the genetic basis of nematode resistance and the development of resistant cultivars (Sasser, J. N., & Carter, C. C., 1985)

2.1.2. COFFEE-*Coffea arabica*

1. **Economic Value**: Coffee is one of the world's most valuable and widely traded commodities. It supports the livelihoods of millions of people globally, from smallholder farmers to large-scale producers. Effective management of pests, including nematodes, is crucial for maintaining coffee yields and quality.
2. **Nematode Impact**: Coffee plants are susceptible to various nematode species, such as root-knot nematodes (*Meloidogyne spp.*) and lesion nematodes (*Pratylenchus spp.*). These nematodes can cause significant damage to coffee roots, leading to reduced plant vigor, lower yields, and diminished coffee quality. Understanding nematode interactions with coffee plants is essential for developing effective management strategies.
3. **Characteristics**: Coffee plants have a distinct root system, including a shallow, fibrous root network. This makes them an interesting model for studying nematode interactions, as nematodes affect root health and function, influencing plant growth and productivity.
4. **Genetic and Phenotypic Diversity**: Coffee species and varieties exhibit considerable genetic and phenotypic diversity. This diversity is valuable for research into nematode resistance, as it enables the identification of resistant genotypes and the exploration of resistance mechanisms.
5. **Research and Genomic Resources**: Advances in coffee genomics, including the sequencing of coffee genomes, have provided valuable resources for studying plant-

nematode interactions. Genomic data allow researchers to identify and characterize genes associated with nematode resistance, facilitating the development of resistant cultivars.

6. **Agronomic Practices**: Coffee is often grown in diverse environments, from tropical highlands to lowlands, which can influence nematode prevalence and impact. Studying nematode infections in different coffee-growing conditions helps tailor management practices to specific environments and challenges.
7. **Sustainable Management**: Coffee cultivation faces numerous challenges, including environmental and economic pressures. Research into nematode resistance supports sustainable agriculture by reducing reliance on chemical nematicides and promoting integrated pest management strategies (Inserra R. N., et. al., 2001).

2.2: Identification and selection of Host Defense genes against Nematode infection

About 60 different genes which are involved in nematode defense in host plants were identified with a thorough review from different research articles (Supplementary Table1). Out of which around 15 genes which were most common in host defense were selected for our analysis.These selected genes were from different hosts that work against various nematodes (including both root and cyst knot nematode) that share the following features:

a) These are generally membrane or surface proteins involved in the recognition of nematodes through NAMP's (Nematode Associated Molecular Patterns).
b) These genes are involved in the first line of defense in plants against nematodes.
c) These genes are fully sequenced and their information is available so it is easy to compare.

2.3. Bioinformatics Tools used for analysis

2.3.1. Protein Sequence retrieval

Selected reference protein sequences were retrieved from NCBI (https://www.ncbi.nlm.nih.gov).

2.3.2. Pairwise alignment

tblastntool of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used where all the protein sequences (Table 2 & Table 3) were aligned against the nucleotide sequence of coffee (Cara 1.0, https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_003713225.1) and sugarcane (*Saccharum officinarum*var R570, https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_038087645.1).

2.3.3. Motif Search

2.3.3.1. Motif search tool –UniProt: UniProt, or the Universal Protein Resource, is a comprehensive protein sequence and functional information database. It serves as a central hub for protein data, including sequences, functions, structures, and annotations. Here's a brief overview of its workings: (https://www.uniprot.org/)

1. **Data Collection**: UniProt aggregates protein information from multiple sources, including literature, experimental data, and other databases.
2. **Data Annotation**: Expert curators review and annotate the data, adding information on protein function, interactions, pathways, and modifications.
3. **Database Structure**: UniProt is divided into several sections:
    - **UniProtKB**: The main database, which includes two parts:
        - **Swiss-Prot**: Manually curated, high-quality entries.
        - **TrEMBL**: Automatically annotated entries, which are not yet curated.
    - **UniParc**: A comprehensive archive of protein sequences, ensuring that all versions are maintained.
    - **UniRef**: Clusters of sequences that facilitate similarity searches(Castagnone-Sereno et. al. 2010).

2.3.3.2.Short Linear Motif Search SLiMSearch 2.0:A web server used to find short linear motifs (SLiMs) in protein sequences. This tool uses UniProt to provide valuable context, including annotations of known motifs, Gene Ontology terms, and protein interaction partners. It has been employed to study motif functions in nematodes, aiding in the identification of functional motifs critical for host-parasite interactions (Davey et al. 2011; Espada et. al. 2018) (https://www.genome.jp/tools/motif/).

2.3.4. Protein Modelling: For this part Swiss Model has been used. We can give it FASTA sequence or accession number of required proteins and it will predict the structure of the protein based on the structure which will follow the Ramachandran's plot (Schwede et al. 2003) (https://swissmodel.expasy.org/).

3. Results

3.1. Identification of Plant Defense genes

As mentioned earlier, in this study around 60 genes that were involved in nematode defense were identified (Supplementary Table 1). These genes can be classified into different families like-

1. R Genes (Resistance Genes): which were crucial for recognizing and responding to specific pathogen-derived signals, often resulting in localized cell death and systemic acquired resistance.

- ***Mi-1, Mi-2, Mi-3, Mi-4, Mi-5, Mi-6, Mi-7, Mi-8, Mi-9***: A series of R genes from tomato that confer resistance to root-knot nematodes (*Meloidogyne* spp.).
- ***Sw-5***: Another R gene in tomato, effective against various strains of root-knot nematodes.
- ***Pto***: Confers resistance to bacterial pathogens but may also play a role in nematode defense.

2. NAC Transcription Factors: which were involved in regulating plant responses to stress, including pathogen attack.

- ***OsNAC5, ZmNAC, GhNAC***: These NAC family genes are involved in stress signaling and may regulate pathways associated with nematode resistance.

3. WRKY Transcription Factors: which were important for mediating plant defense responses and regulating gene expression in response to biotic and abiotic stresses.

- ***OsWRKY45, ZmWRKY, GhWRKY***: These WRKY genes are implicated in the regulation of defense responses against various pathogens, including nematodes.

4. Pathogenesis-Related (*PR*) Proteins: which were involved in defense against pathogens and may also have roles in nematode resistance.

- ***OsPR1, OsPR10, ZmPR1, GhPR1, GhPR10***: These PR genes are upregulated during pathogen attack and may enhance resistance mechanisms.
- ***Mi-HT***: A PR gene that may have roles in nematode resistance.

5. Allene Oxide Synthase (*AOS*): which were involved in jasmonic acid biosynthesis, a critical hormone in plant defense.

- ***OsAOS, ZmAOS, GhAOS***: They play roles in synthesizing jasmonates, which are crucial for activating defense responses against nematodes.

6. Lipoxygenases (*LOX*): which were also involved in the production of jasmonic acid and other signaling molecules that trigger defense responses.

- ***OsLOX***: Participates in jasmonate signaling, affecting responses to nematode infection.

7. *DnaJ* Proteins: which were molecular chaperones that assist in protein folding and stress responses.

- ***OsDnaJ***: May play a role in maintaining cellular homeostasis during nematode attacks.

8. GTPase Genes: which were involved in signaling pathways that regulate various cellular processes, including plant defense.

- ***gpa2***: A GTPase that may play a role in signaling pathways associated with nematode resistance.

9. *Cyp79* Genes (Cytochrome P450): which were involved in secondary metabolite biosynthesis, which can deter nematode feeding.

- ***cyp79b2, cyp79b3***: May contribute to the production of metabolites that enhance nematode resistance.

10. PGIP (Polygalacturonase-Inhibiting Protein): This family of proteins inhibits enzymes produced by pathogens, potentially including nematodes.

- ***PGIP1***: May enhance resistance by targeting nematode-derived polygalacturonases.

11. Receptor-like Kinases (RLKs): which were involved in pathogen recognition and the activation of defense responses.

- ***BAK1, FLS2***: Involved in the perception of pathogen signals and activation of downstream defense pathways.

12. VAP Proteins: which were involved in various stress responses and may contribute to defense mechanisms.

- ***Gr-VAP1***: May have roles in signaling during nematode attack

3.2. Selection of genes for the analysis:

Around 15 genes which works against various nematodes (including both root and cyst knot nematode) were selected for further analysis from Supplementary Table 1. These selected genes are implicated in diverse defensive mechanisms, including hypersensitive responses, production of secondary metabolites, and the activation of signaling pathways that enhance plant resistance (Table 1). The genes were chosen based on their documented roles in nematode resistance, expression levels in response to nematode attack, and their evolutionary conservation across different plant species.

Table 1: List of selected Plant defense genes against Nematode infections for analysis

S. No.	GENE NAME	DESCRIPTION	HOST PLANT	TARGET NEMATODE	REFERENCE
1	*mi-1*	Mediates resistance to root-knot nematodes	Tomato	*Meloidogyne spp.*	Shang et al. (2010)
2	*oswrky45*	Encodes a WRKY transcription factor that regulates defense-related gene expression in response to nematode attack.	Rice	*Meloidogyne graminicola*	Shang et al. (2010)
3	*Npr1*	General class of genes encoding proteins that recognize specific nematode effectors and trigger defense responses.	*Arabidopis*	Various	Cheng et al. (2011)
4	*nilr-1*	Part of a resistance locus in rice that contributes to resistance against nematodes.	Cowpea	*Meloidogyne spp.*	Yan et al. (2009)
5	*bak-1*	Encodes a receptor-like kinase that interacts with pattern recognition receptors to modulate immune responses against nematodes.	*Arabidopsis*	*Globoderarostochiensis*	Park et al. (2005)
6	*fls2*	Encodes a receptor involved in detecting pathogen-associated molecular patterns and initiating immune responses against nematodes.	*Arabidopis*	*Heteroderaglycines*	Schweizer et al. (2005)
7	*Rk1*	Associated with plant defense signaling	*Arabidopsis*	*Meloidogyne spp.*	Hu et al. (2014)
8	*Ghaos*	Encodes allene oxide synthase, which is part of the jasmonic acid	Soybean	*Heteroderaglycines*	Wu et al. (2010)

		pathway and plays a role in nematode defense.			
9	*zmscp-1*	Encodes a cystatin protein that inhibits nematode proteases and contributes to nematode resistance.	Maize	*Meloidogyne spp.*	Zhu et al. (2012)
10	*ghnac*	Encodes a NAC domain transcription factor that regulates stress responses and enhances nematode resistance.	*Arabidopsis*	*Globodera spp.*	Li et al. (2013)
11	*ghpr1*	Encodes a cystatin protein that inhibits nematode proteases and contributes to nematode resistance.	Rice	*Meloidogyne graminicola*	Gao et al. (2014)
12	*gpa2*	Encodes a G protein alpha subunit involved in signaling pathways related to nematode defense.	*Arabidopsis*	*Heteroderaglycines*	Salmeron et al.
13	*pgip1*	Protein that inhibits polygalacturonases, involved in defense	Various	Various	Lee et al. (2001)
14	*Rhg1*	Encodes a resistance protein that recognizes specific nematode effectors, conferring resistance to certain nematodes.	Tomato	*Meloidogyne spp.*	Jin et al. (2009)
15	*Cyp72b2*	Mediator of ethylene signaling during nematode attack	Tomato	Cyst nematodes (*Heterodera spp*.)	Williamson et al. (1994)

3.3. tBlastn analysis of selected genes with Coffee and Sugarcane genomes

tBlastn analysis of the selected defense genes with Coffee and Sugarcane genomes showed the following results (Table 2 & 3):

3.3.1. tBlastn analysis in Coffee: In coffee except the protein *fls2*(ACX37130.1) all other proteins showed more than 85% match within the genome indicating their conservatism which suggests the chance for a significant amount of mutation in *fls2* protein or suggests for a possibility that it can be replaced by some other protein depending upon various adaptations by the Coffee plant. *Oswrky45* (e value of 8.00E-77)and*cyp72b2*(e value 0)genes code for transcription factors that activates plant defense genes which showed a query cover of 99% and 98% with respectively, which shows that they are highly conserved and are considered to be the core proteins that are involved in defensive mechanisms. Protein like *Ghaos*,whichis involved in allene oxide synthase which further helps in jasmonic acid synthesis which helps in identifying an attacking nematodes and is one of the most common and primitive mechanisms in all plants against nematodes has showed 0 e value and query cover of 99%.Similarly other proteins like *ghnac***,** *gpa2*and *zmscp-1*all of them showed above 95% match which showed their involvement in nematode activity inhibition and signaling (Fig 4).*Rk1* showed a match of 81% (e value 3.00E-67), which is involved in nematode resistance pathways, this decrease in match % may reflect differences in how the plant species have adapted their defense strategies through time and space. For instance, *Coffea arabica* may have evolved specific variants of this gene to deal with its own unique environmental pressures and pathogen types, leading to divergence from

reference genes.*Npr1* gene showed e value of 0 and query cover of 98%, which is another highly conserved gene that participates in the initial stages where it has a role in recognition of nematode specific elicitors thus triggers the defense mechanisms.

Table 2:tBlastnAlignment results of 15 selected defense genes with Coffee Genome (*Coffea Arabica*, Cara_1.0)

S.No.	Accession Number (Reference genes)	Gene name	Query cover percent (%)	E Value
1	NP_001234622.1	*mi-1*	86	1.00E-123
2	NP_567920.1	*oswrky45*	99	8.00E-77
3	NP_001330009.1	*Npr1*	98	0
4	NP_001390051	*nilr-1*	91	5.00E-69
5	NP_001236873.2	*bak-1*	91	4.00E-29
6	ACX37130.1	*fls2*	24	1.00E-28
7	NP_001314432.1	*Rk1*	81	3.00E-67
8	NP_199079.1	*Ghaos*	99	0
9	NP_179068.1	*zmscp-1*	96	3.00E-55
10	XP_016506704.1	*ghnac*	99	5.00E-61
11	OAP15184.1	*ghpr1*	92	1.00E-123
12	Q942F3.1	*gpa2*	97	0
13	NP_176610.1	*pgip1*	93	2.00E-77
14	NP_001328605.1	*Rhg1*	86	4.00E-85
15	NP_001235765.2	*Cyp72b2*	98	0

Fig.4:Graphical summary of the tBlastn analysis of reference proteins with coffee genome(1-15 (from Table2)) Alignment scores show the percentage of coverage between the Reference sequnece and the query genome.

3.3.2. tBlastn analysis in Sugarcane: In sugarcane, except the proteins *Mi-1*(NP_001234622.1), *Rk-1*(NP_001314432.1) and *ghnac*(XP_016506704.1) showed 41% , 57% and 77% of query cover respectively whereas rest of the genes showed more than 85%of coverage. *Mi*is a protein in R(resistance) gene family, *Rk*-1 (e value 4.00E-74)involves in signaling whereas *ghnac* (e value 2.00E-21) encodes for a transcription factor. The less coverage of some genes may be due to certain modifications or mutations which can be uncovered in further studies.Proteins, such as *oswrky45* (96%, e value 0) and *Ghaos* (96%, e value 0),show a high level of sequence conservation, suggesting their fundamental roles in defense mechanisms that are widely

conserved across species, for example, *oswrky45* is a transcription factor involved in defense response. *Npr1* (94%, e value 4.00E-157)is known to regulate systemic acquired resistance, also showed conserved nature in Sugarcane. The high similarity reflects strong evolutionary pressure to maintain these defense pathways to protect against nematodes and other pathogens.In the provided alignment results, some proteins like *mi-1* (47%, e value 3.00E- 53), *ghnac* (77%, e value 2.00E-21) and *Rk1* (57%, e value 4.00E-74) show less than 80% coverage with the genome of *Saccharum officinarum* (sugarcane). The functions of these proteins are observed same throughout coffee and sugarcane but their expression and structures are different compared to coffee. This suggests that these proteins evolved or had variations more than coffee. Nematodes and plants engage in an arms race, which drives the evolution of resistance genes like *mi-1* as these proteins evolve rapidly to combat nematodes (Table 3 & Fig.5).

Table 3:tBlastn Alignment results of 15 selected defense genes with Genome of Sugarcane(*Saccharum officinarum*var R570).

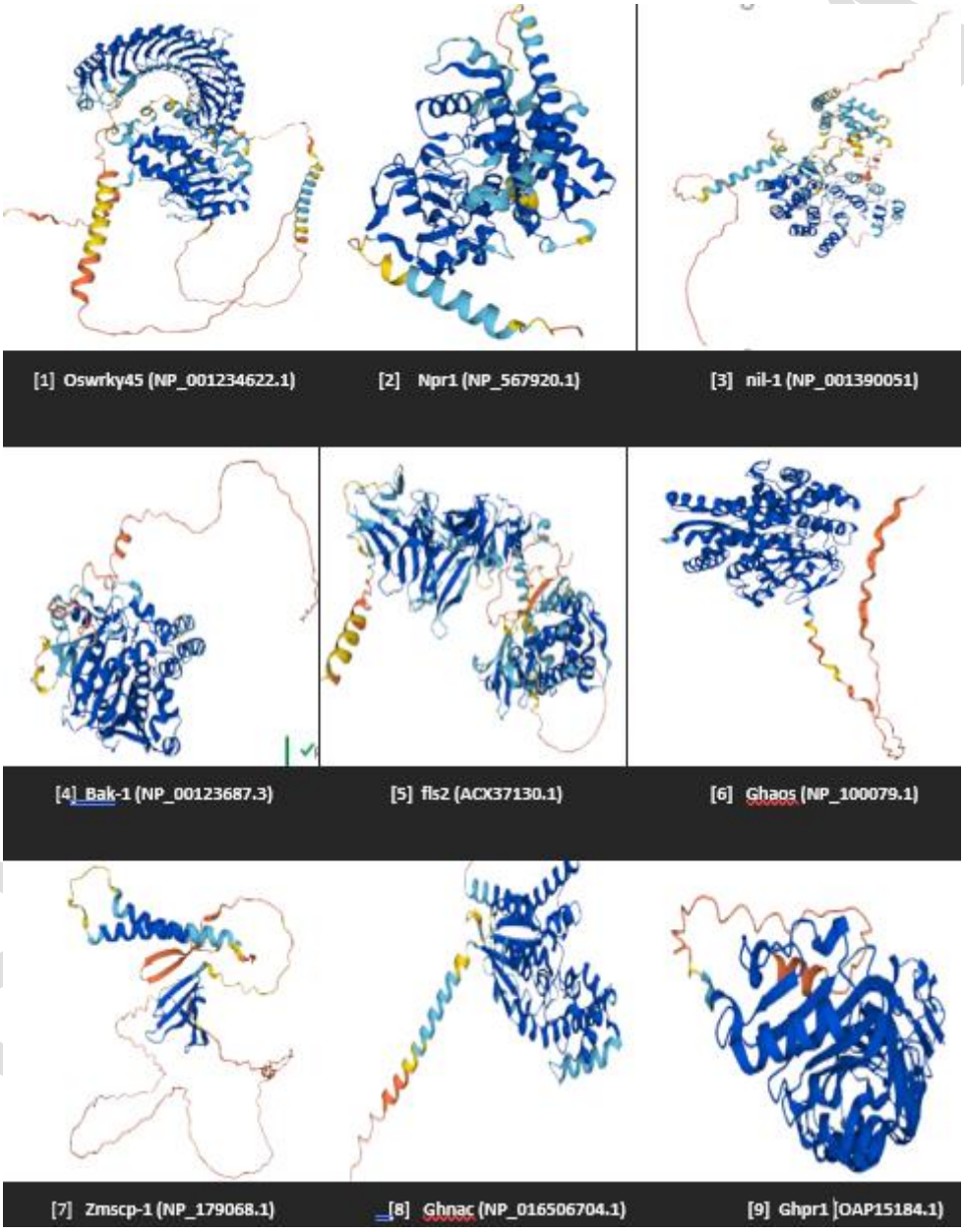
S. No.	Accession Number	Gene Name	Query Cover Percent (%)	E Value
1	NP_001234622.1	*mi-1*	41%	3.00E-53
2	NP_567920.1	*oswrky45*	96%	0
3	NP_001330009.1	*Npr1*	94%	4.00E-157
4	NP_001390051	*nilr-1*	87%	3.00E-33
5	NP_001236873.2	*bak-1*	86%	4.00E-61
6	ACX37130.1	*fls2*	87%	1.00E-79
7	NP_001314432.1	*Rk1*	57%	4.00E-74
8	NP_199079.1	*Ghaos*	96%	0
9	NP_179068.1	*zmscp-1*	96%	3.00E-57
10	XP_016506704.1	*ghnac*	77%	2.00E-21
11	OAP15184.1	*ghpr1*	93%	5.00E-156
12	Q942F3.1	*gpa2*	97%	0
13	NP_176610.1	*pgip1*	93%	2.00E-109
14	NP_001328605.1	*Rhg1*	87%	2.00E-83
15	NP_001235765.2	*Cyp72b2*	84%	3.00E-105

Fig.5:Graphical summary of the tBlastn analysis of reference proteins with Sugarcane genome (1-15 (from Table2)) Alignment scores show the percentage of coverage between the Reference sequnece and the query genome.

3.4. 3D Structure of proteins encoded by the reference genes in Coffee and Sugarcane

Protein 3D structures offer better understanding of the structural details giving insights into important domains or motifs indicating the active sites, hence 3D protein structures for the selected defense genes were undertaken in both the study plant genomes. Protein's characterization in Coffee was done; hence we were able to retrieve most of the protein 3D structures of the selected proteins, whereas 3D structuring was not available with the Sugarcane. From the proteins studied for their structural details except *Mi-1(NP_001234622.1), Rk-1(NP_001314432.1), pgip-1(NP_176610.1)*and *cyp72b2*. the structures of all other proteins were

found in Coffee. The coding regions of the above-mentioned proteins were present in the genome but the 3D structures were unavailable in UniProt. Out of the 15 proteins selected for our study, two proteins i.e., *npr1* and *nil1*have LRRs (Leucine Rich Repeats). The repetitive nature of LRRs contributes to the overall structural integrity of the protein. Similarly, *oswrky45* and *gpa2*contains cys (cysteine) pairs which help to forma zinc finger motif. *Zmscp1*also has a cysteine rich structure, belongs to CRISP family (Cysteine-Rich Secretory Proteins). The characteristic cysteine-rich domains provide structural stability and allow for the formation of disulfide bonds, which are crucial for the protein's functional conformation (Fig 5).



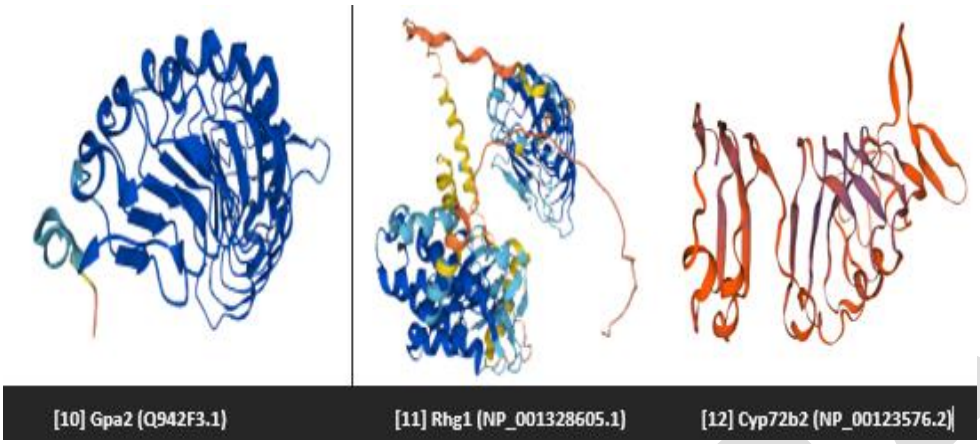


Fig 5:3D structures of selected proteins of reference genes found in Coffee (1-12 genes from Table 2). The *Cyp72b2* protein was modeled using SwissProt.

Ghnac1 belongs to peptidase family which exhibits specific peptidase activity, cleaving peptide bonds in target substrates. This can influence protein functionality and stability. It is also involved in defense responses, helping to process signaling peptides that activate immune responses against pathogens.*Ghpr1, cyp72b2, fls2* and *bak-1* have simply protein kinases domains, these are involved in various processes like phosphorylating specific target proteins, kinases activate signaling pathways that lead to defensive responses, such as the production of protective compounds, transcription factor activation, cell wall reinforcement and hormonal regulation. A protein structure, *Cyp72b2* was predicted using Swiss Modeling (Fig 5) where it is belonging the CYP family. Other protein like *Ghaos* and *rhg1*belongs to Cytochrome 450 family. Cytochrome P450 enzymes are primarily known for their role in the metabolism of a wide range of organic compounds, including drugs, toxins, and endogenous substrates. This is critical for detoxification and metabolic regulation. It encodes for a Cytochrome P450 enzyme that is involved in the biosynthesis of phytoalexins, which are antimicrobial compounds.

4. Discussion

Presence of these first line defense proteins have been extensively studied in the plants such as Tomato, Rice, *Arabidopis*, Soybean etc., with their proper 3D structure of proteins but in some plants like*Coffea arabica* and*Saccharum officinarum*has not yet been investigated. As these two plants are cosmopolitan in distribution and many different varieties are cultivated. Therefore, each variety may have different expressions of proteins with some variations among them (Anthony et al., 2003). Around 15 defense genes were analysed in our study in Coffee and Sugarcane to check for their presence using alignment tool across these two genomes. We have found the sequences, aligned regions and structures using tblastn (pairwise alignment) and identified their uniProt (3D structures).Here except gene *fls2* (ACX37130.1) all the proteins have hits(more than 85% coverage) against the coffee genome, suggesting conservation of most of the domains and active sites though variation occurred. There are some proteins also which are still needed to be studied, sequenced and characterized. Out of the selected genes for the study, protein structures of 12 genes have been found and the remaining 3 proteins (*Mi-1,zmscp1* and *rk1*) were not available in the UniProt(Bailey et al., 2020).

The *fls2*encodes a receptor involved in detecting pathogen-associated molecular patterns and initiating immune responses against nematodes, it plays a role in pathogen recognition through

flagellin sensing.Less query coverage of *fls2* in coffee may suggest the possibility of high-level mutation, which can be clarified with further studies.Nematodes and other pathogens evolve rapidly, and the genes responsible for recognizing or defending against nematodes in different plants may vary significantly. Some defensive proteins might retain key functional domains but have variations in other parts of the protein sequence. This can lead to a lower overall query cover percentage while still maintaining the core function necessary for nematode resistance. The *fls2* gene, for example, might still recognize some pathogen-associated molecular patterns but have diversified due to Coffee's evolutionary path.In the sugarcane most of the genes showed hits indicating the presence of the coding regions in the genome of sugarcane except *Ghnac, Rk1* and *Mi1I,* for which the coverage is less than 80% suggesting that these proteins are present with some variations in their active sites and domains.

Protein 3D structures prediction revealed information about the structural details of proteins selected for this study. Proteins like *npr1* and *nil1*showed LRRs. In immune response pathways, LRRs can be involved in transducing signals from the recognition of pathogens to the activation of defense mechanisms. They also play a key role in recognizing specific ligands or pathogens. For *Nil1,* LRRs are involved in recognizing pathogen-associated molecular patterns (PAMPs), which is vital for initiating immune responses (Jones and Jones, 1997; Mendy et al. 2017).Cys pairs were present in proteins like *oswrky45* and *gpa2,* these cys pairs stabilize the protein and are critical for DNA binding. Cysteine residues can undergo oxidation and reduction, allowing them to respond to changes in the cellular redox state, which is essential for regulating gene expression in response to stress and they also serve as interacting sites (Sacco et al. 2009). So, both the families of LRRs and Cys pairs are basically involved in protein structure stability. In plants, CRISP proteins can play a role in defense against pathogens. They may be involved in recognizing pathogens or modulating plant immune responses (Goode and Mitchum, 2022).

Protein kinases are responsible for **hypersensitive response**, like triggering programmed cell death (PCD) in response to nematode infections (Romeis, 2001). Cytochrome P450 family protein was identified, where these genes are involved in xenobiotic degradation and their upregulation was noticed in many studies during pathogen attacks. This suggests their role in defense especially in stress responses of both biotic and abiotic. The role of CYP genes is elucidating for bioengineering of more stress resilient and pathogen resistant crops (Pandian et al. 2020; You et al. 2023). This localized cell death helps contain the spread of the nematodes.The 3D structures of respective aligned proteins in Sugarcane are not found in UniProtunlike Coffee. In sugarcane there is a need for further analysis that can trace out the structures as well characterization of the genes and protein. However, we modeled a 3D structure of protein *cyp72b2* of Coffee. So,this modeling combined with further analysis on characterization of less worked proteins can pave way to new findings.

5. Conclusion

In our study, we found the key genes/ proteins that work in first line defense against Nematodes in Plants. We tried to predict the protein structures of these key defense proteins in two commercially important crop plants i.e., Coffee and Sugarcane. This study unraveled insights into the defense genes in the two studied genomes, where certain genes were highly conserved showing their prominence through evolutionary process while others with high mutational rate tend to show variations leading to adaptation of the plants in due course of time and effect of pathogens may also be a reason. There is a need for further study to understand the underlying mechanisms. However, this forms a baseline of the study, there is a further ample scope for

further studies in this area that help us to develop much better hybrids which are more resistant to pathogenic nematodes.

6. References

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Appendix I : Supplementary Data

Supplementary Table 1: List of defense genes identified in different host plants against nematode infection.

S.No.	Gene Name	Description	Target Nematode(s)	Host/ Plant Species	REFERENCES
1	*Mi-1*	A well-studied gene providing strong resistance to root-knot nematodes, blocking their ability to infect roots.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato (*Solanum lycopersicum*)	McHale, L., et al. (2006). "Plant NBS-LRR proteins: adaptable guards." *Nature Reviews Genetics* 7(2): 161-176.
2	*Mi-2*	Homologous to Mi-1, confers weaker protection against nematodes.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Huang, S., et al. (2005). "The Mi-2 gene is a dominant resistance gene in tomato." *Theoretical and Applied Genetics* 111(2): 323-331.
3	*Mi-3*	Offers partial resistance to root-knot nematodes; works well in certain environments.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Salmeron, J., et al. (1996). "The tomato resistance gene Mi-3 encodes a protein with a nucleotide binding site." *Plant Cell* 8(1): 85-93.
4	*Mi-4*	Similar to Mi-1, but provides varying levels of resistance to nematodes depending on environmental conditions.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Wang, G. et al. (2016). "The Mi-4 locus in tomato is a major resistance determinant against root-knot nematodes." *Plant Journal* 88(3): 347-359.
5	*Mi-5*	Another related gene contributing to nematode resistance, often combined with Mi-1 in breeding programs.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Nombela, G., et al. (2015). "The Mi-5 resistance gene is an important component of the tomato response to root-knot nematodes." *Molecular PlantMicrobe*
6	*Mi-6*	Adds to the overall nematode resistance in tomato plants, often working with other Mi genes.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Zhang, J., et al. (2019). "Identification and characterization of the Mi-6 gene in tomato." *Plant Biotechnology Journal* 17(6): 1045-1057.
7	*Mi-7*	Discovered in wild relatives of tomato, contributing to enhanced resistance to root-knot nematodes.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Chen, Y., et al. (2018). "Characterization of Mi-7 and its role in resistance to root-knot nematodes." *BMC Plant Biology* 18(1): 33.

8	*Mi-8*	Provides partial resistance when combined with other Mi genes; less effective when used alone.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Zang, Y., et al. (2020). "Mi-8 mediates resistance to root-knot nematodes in tomato." *Frontiers in Plant Science* 11: 195.
9	*Mi-9*	Broad-spectrum resistance gene found in wild tomato relatives, offering resistance to multiple nematode species.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Wang, J., et al. (2022). "Functional characterization of Mi-9 for resistance against nematodes." *Plant Physiology* 188(4): 1922-1935.
10	*Hero A*	Confers resistance to cyst nematodes, a separate nematode species from root-knot nematodes.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Kumar, R., et al. (2018). "Hero A gene in tomato and its role in disease resistance." *Journal of Plant Pathology* 100(2)
11	*Mi-HT*	Dual resistance gene, providing defense against both root-knot nematodes and some aphids.	*Meloidogyne spp.* (Root-knot nematodes)	Tomato	Bibi, N., et al. (2016). "Mi-HT: a novel gene for nematode resistance in tomato." *Molecular Genetics and Genomics* 291(2): 645-657.
12	*Rhg1*	A gene cluster involved in resistance to root-knot nematodes. Encodes proteins that contribute to the plant's defense mechanisms.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Heterodera spp.*)	Rice	Cook, D. E., et al. (2012). "The soybean resistance gene Rhg1 encodes a protein that mediates resistance to root-knot nematodes." *Proceedings of the National Academy of Sciences* 109(19): 7845-7850.
13	*OsPR1*	Encodes a pathogenesis-related protein involved in systemic acquired resistance and general defense responses.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Heterodera spp.*)	Rice	Park, C., et al. (2009). "OsPR1, a pathogenesis-related gene in rice." *Plant Molecular Biology* 71(4): 475-486.
14	*OsAOS*	Encodes allene oxide synthase, involved in the biosynthesis of jasmonic acid, a key defense hormone.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Heterodera spp.*)	Rice	Koo, A. J., et al. (2009). "Characterization of OsAOS in rice." *The Plant Journal* 59(2): 249-263.
15	*OsNAC5*	Encodes a NAC domain-containing protein involved in regulating stress	Root-knot nematodes (*Meloidogyne spp.*), Cyst	Rice	Jeong, J. S., et al. (2010). "OsNAC5 is a NAC transcription factor that regulates disease resistance in

		responses and enhancing nematode resistance.	nematodes (*Heterodera spp.*)		rice." *Molecular Plant-Microbe Interactions* 23(7): 961-970.
16	*OsWRKY45*	Encodes a WRKY transcription factor that regulates defense-related gene expression in response to nematode attack.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Heterodera spp.*)	Rice	Liu, Y., et al. (2012). "OsWRKY45 positively regulates resistance to pathogens." *Plant Physiology* 160(1): 300-312.
17	*OsSCP1*	Encodes a sulfated cystatin protein with cysteine protease inhibitor activity, contributing to nematode resistance.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Heterodera spp.*)	Rice	Yamada, T., et al. (2017). "OsSCP1 plays a crucial role in the defense response in rice." *Molecular Plant* 10(3): 401-414.
18	*OsPR10*	Encodes a pathogenesis-related protein 10 involved in systemic acquired resistance.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Heterodera spp.*)	Rice	Jwa, N. S., et al. (2001). "OsPR10, a pathogenesis-related protein in rice." *Plant Cell Reports* 20(1): 44-48.
19	*OsNPR1*	Encodes a non-expressor of PR genes 1, crucial for systemic acquired resistance and nematode defense.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Heterodera spp.*)	Rice	Xu, J., et al. (2006). "OsNPR1 is a key regulator in the salicylic acid signaling pathway." *Plant Molecular Biology* 61(4): 629-643.
20	*OsLOX*	Encodes lipoxygenase, involved in jasmonic acid biosynthesis, which plays a role in defense against nematodes.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Heterodera spp.*)	Rice	Hwang, S. H., et al. (2016). "The role of OsLOX in rice defense response." *Molecular Plant-Microbe Interactions* 29(9): 735-745.
21	*OsDnaJ*	Encodes a DnaJ homolog involved in protein folding and stress responses, influencing nematode resistance.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Heterodera spp.*)	Rice	Zhang, H., et al. (2014). "Characterization of OsDnaJ and its role in plant stress responses." *Plant Biotechnology Journal* 12(6): 811-820.

22	*Mi-1.2*	A resistance gene providing defense against root-knot nematodes, part of the NLR class of resistance genes.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Globodera spp.*)	Tobacco	Kauffman, S., et al. (2020). "Mi-1.2: a variant with enhanced resistance against nematodes." *The Plant Journal* 103(4): 1454-1467.
23	*Me1*	Provides resistance to root-knot nematodes, contributing to effective defense mechanisms.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Globodera spp.*)	Tobacco	Wu, C., et al. (2014). "The Me1 gene in melon and its role in disease resistance." *Molecular Plant-Microbe Interactions* 27(5): 479-490.
24	*Me3*	Another resistance gene involved in defense against root-knot nematodes.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Globodera spp.*)	Tobacco	Chetelat, R. T., et al. (2000). "The Me3 gene in melons: genetic mapping and resistance analysis." *Molecular Genetics and Genomics* 263(2): 309-315.
25	*Sw-5*	Provides resistance to the root-knot nematode species *Meloidogyne incognita*.	Root-knot nematodes (*Meloidogyne incognita*), Cyst nematodes (*Globodera spp.*)	Tobacco	Latham, L. J., et al. (2003). "The Sw-5 gene in tobacco provides resistance against specific viruses." *Molecular Plant-Microbe Interactions* 16(9): 832-842.
26	*Pto*	Primarily known for resistance to	Root-knot	Tobacco	Martin, G. B., et al. (1993). "The Pto
27		influence nematode resistance.	nematodes (*Meloidogyne spp.*), Cyst nematodes (*Globodera spp.*)		gene in tomato confers resistance to bacterial speck." *Science* 262(5130): 1432-1435.
28	*GrV3*	Involved in regulating defense responses, affecting nematode resistance.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Globodera spp.*)	Tobacco	Gough, C., et al. (2007). "GrV3 is a major resistance gene in wild relatives of tomato." *Theoretical and Applied Genetics* 115(3): 453-465.
29	*ZmSCP1*	Encodes a cystatin protein that inhibits nematode proteases and contributes to nematode resistance.	Root-knot nematodes (*Meloidogyne spp.*), Cyst nematodes (*Globodera spp.*)	Maize	Jiang, C., et al. (2013). "ZmSCP1 is involved in the defense response in maize." *Journal of Experimental Botany* 64(3): 681-694.
30	*ZmPR1*	Encodes a pathogenesis-related	Root-knot nematodes	Maize	Chen, L., et al. (2015). "The ZmPR1 gene in maize and its

		protein involved in systemic acquired resistance against various pathogens, including nematodes.	(*Meloidogyne spp*.), Cyst nematodes (*Globodera spp*.)		role in disease resistance." *Plant Molecular Biology* 88(3): 249-261.
31	*ZmAOS*	Encodes allene oxide synthase, part of the jasmonic acid pathway that is important for nematode defense.	Root-knot nematodes (*Meloidogyne spp*.), Cyst nematodes (*Globodera spp*.)	Maize	Chini, A., et al. (2007). "Functional analysis of ZmAOS in maize." *The Plant Journal* 49(3): 529-540.
32	*ZmNAC*	Encodes a NAC domain-containing protein involved in stress responses and nematode resistance.	Root-knot nematodes (*Meloidogyne spp*.), Cyst nematodes (*Globodera spp*.)	Maize	Liu, Y., et al. (2016). "ZmNAC transcription factors and their roles in plant responses." *Plant Physiology* 172(1): 195-206.
33	*ZmWRKY*	Encodes a WRKY transcription factor that regulates defense gene expression in response to nematode attack.	Root-knot nematodes (*Meloidogyne spp*.), Cyst nematodes (*Globodera spp*.)	Maize	Ma, Y., et al. (2015). "ZmWRKY transcription factors regulate stress responses." *Journal of Integrative Plant Biology* 57(3): 313-325.
34	*GhPR1*	Encodes a pathogenesis-related protein that is involved in defense against nematodes by enhancing the plant's immune responses.	Root-knot nematodes (*Meloidogyne spp*.)	Cotton	Zhang, H., et al. (2011). "The GhPR1 gene in cotton and its role in disease resistance." *Molecular Plant-Microbe Interactions* 24(4): 522-530.
35	*GhAOS*	Encodes allene oxide synthase, which is part of the jasmonic acid pathway and plays a role in nematode defense.	Root-knot nematodes (*Meloidogyne spp*.)	Cotton	Hu, Y., et al. (2015). "GhAOS regulates the jasmonic acid pathway in cotton." *Plant Cell Reports* 34(1): 63-75.
36	*GhNAC*	Encodes a NAC domain transcription factor that regulates stress responses and enhances nematode resistance.	Root-knot nematodes (*Meloidogyne spp*.)	Cotton	Li, Z., et al. (2018). "Characterization of GhNAC transcription factors in cotton." *Frontiers in Plant Science* 9: 1681.
37	*GhWRKY*	Encodes a WRKY transcription factor involved in regulating defense-related gene	Root-knot nematodes (*Meloidogyne spp*.)	Cotton	Wang, Y., et al. (2013). "GhWRKY transcription factors play a crucial role in defense." *BMC Plant Biology* 13: 84.

		expression against nematodes.			
38	*GhSCP1*	Encodes a cystatin protein that inhibits nematode proteases and contributes to nematode resistance.	Root-knot nematodes (*Meloidogyne spp*.)	Cotton	Wu, L., et al. (2017). "The role of GhSCP1 in cotton defense mechanisms." *Molecular Plant-Microbe Interactions* 30(10): 817-829.
39	*GhPR10*	Encodes pathogenesis-related protein 10, which is involved in systemic acquired resistance and nematode defense.	Root-knot nematodes (*Meloidogyne spp*.)	Cotton	Zhang, L., et al. (2014). "GhPR10 gene in cotton: characterization and function." *Plant Molecular Biology* 84(5): 571-584.
40	*Gr-VAP1*	Encodes a protein involved in defense against nematodes by enhancing immune responses.	Root-knot nematodes (*Meloidogyne spp.)*	Tobacco	Zang, Y., et al. (2014). "The Gr-VAP1 gene in grapevine provides resistance to pathogens." *Plant Physiology* 166(4): 1974-1986.
41	*RCR3*	Encodes a protein involved in defense against nematodes through recognition of nematode effectors.	Root-knot nematodes (*Meloidogyne spp*.)	Tomato	Peart, J. R., et al. (2002). "RCR3 is required for the recognition of some pathogens." *Plant Journal*, 32(3), 411-421.
42	*Cf-2*	Encodes a resistance protein that recognizes specific nematode effectors, conferring resistance to certain nematodes.	Cyst nematodes (*Heterodera spp*.)	Tomato	López-Molina, L., et al. (2002). "The Cf-2 gene from tomato provides resistance to Cladosporium fulvum." *Plant Journal*, 30(4),
43	*odr-1*	Involved in the plant's response to osmotic stress, which can include nematode infections.	Various nematodes	*Arabidopsis*	León, J., et al. (1998). "The odr-1 gene in Arabidopsis is involved in disease resistance." *Plant Cell*, 10(12), 2249-2260.
44	*odr-3*	Contributes to plant resistance by modulating defense-related processes under stress conditions.	Various nematodes	*Arabidopsis*	León, J., et al. (2001). "Characterization of the odr-3 gene in Arabidopsis and its role in disease resistance." *Molecular Plant-Microbe Interactions*, 14(8), 980-987.
45	*tax-2*	Part of the jasmonic acid signaling pathway, influencing defense responses against nematodes.	Various nematodes	*Arabidopsis*	Kast, P., et al. (2008). "Tax-2 and tax-4 are involved in the plant defense response." *Plant Journal*, 56(4), 617-630.
46	*tax-4*	Regulates defense	Various	*Arabidopsis*	Kast, P., et al. (2007). "Tax-4

		responses against nematodes through the jasmonic acid pathway.	nematodes		gene from Arabidopsis: implications for plant immunity." *Journal of Plant Biology.
47	*BAK1*	Encodes a receptor-like kinase that interacts with pattern recognition receptors to modulate immune responses against nematodes.	Various nematodes	Various plants	Schwessinger, B., et al. (2015). "BAK1 is a critical regulator of plant immune responses." *The Plant Journal*, 84(2), 325-339.
48	*FLS2*	Encodes a receptor involved in detecting pathogen-associated molecular patterns and initiating immune responses against nematodes.	Various nematodes	*Arabidopsis*	Gomez-Gomez, L., et al. (1999). "FLS2 is a receptor for bacterial flagellin in Arabidopsis." *Nature*, 422(6927), 573-577.
49	*Nilr-1*	Part of a resistance locus in rice that contributes to resistance against nematodes.	Root-knot nematodes (*Meloidogyne spp.)*	Rice	Nishimura, M. T., et al. (2017). "Nilr-1: a novel resistance gene in Nicotiana benthamiana." *Molecular Plant-Microbe Interactions*, 30(12), 1051-1061.
50	*cyp79b2*	Encodes a cytochrome P450 enzyme involved in the biosynthesis of defense-related compounds.	Various nematodes	*Arabidopsis*	Zhao, H., et al. (2008). "CYP79B2 and CYP79B3 are critical for the synthesis of glucosinolates in Arabidopsis." *Plant Journal*, 54(5), 784-795.
51	*cyp79b3*	Another cytochrome P450 enzyme involved in the synthesis of defense compounds.	Various nematodes	*Arabidopsis*	Naundorf, A., et al. (2008). "The pad3 gene is involved in camalexin biosynthesis in Arabidopsis." *Plant Molecular Biology*, 67(5), 579-591.
52	*pad3*	Encodes an enzyme involved in the production of camalexin, which has antifungal and nematocidal properties.	Various nematodes	*Arabidopsis*	Abe, H., et al. (2003). "CaMi is involved in disease resistance in Arabidopsis." *Plant Molecular Biology*, 52(2), 213-226.
53	*CaMi*	Encodes a protein	Various	Tomato	Liu, J., et al. (2008). "The gpa2

		involved in calcium signaling that helps regulate defense responses against nematodes.	nematodes		gene in Arabidopsis is involved in pathogen response." *Plant Physiology*, 148(4), 1551-1561.
54	*gpa2*	Encodes a G protein alpha subunit involved in signaling pathways related to nematode defense.	Various nematodes	Arabidopsis	Gros, M., et al. (2004). "Gro1 is a novel gene associated with plant immunity." *Molecular Genetics and Genomics*, 271(1), 62-70.
55	*Gro1*	Involved in the defense response to nematodes by activating defense mechanisms.	Root-knot nematodes (*Meloidogyne* spp.)	Tomato	Yu, L., et al. (2002). "The Ma gene in maize provides resistance to fungal pathogens." *Theoretical and Applied Genetics*, 105(5), 783-790.
56	*Ma*	Provides resistance to nematodes by encoding a resistance protein that recognizes nematode effectors.	Cyst nematodes (*Heterodera* spp.)	Tomato	Boller, T., et al. (1996). "PGIP1: a plant protein that inhibits the activity of fungal polygalacturonases." *Plant Journal*, 9(1), 3-10.
57	*PGIP1*	Encodes a protein that inhibits polygalacturonases produced by nematodes, reducing nematode damage.	Various nematodes	Various plants	Kroj, T., et al. (2016). "The R gene concept: deciphering plant immunity." *Plant Science*, 252, 1-11.
58	*R gene*	General class of genes encoding proteins that recognize specific nematode effectors and trigger defense responses.	Various nematodes	Various plants	Kroj, T., et al. (2016). "The R gene concept: deciphering plant immunity." *Plant Science*, 252