Research Article

Morpho-Molecular Characterization of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Tylenchida: Meloidogynidae) from *Solanum melongena* L. (Solanales: Solanaceae)

Rishil Gupta[1], Faheem Ahmad[1]*, Khalid Z Masoodi[2] and Adnan Shakeel[3]

[1]Department of Botany, Aligarh Muslim University, Aligarh-202002, India; [2]Transcriptomics Laboratory (K-Lab) Division of Plant Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), Srinagar 190025, India; [3]CSIR-Indian Institute of Integrative Medicine, Jammu, India.

Rishil Gupta and Faheem Ahmad contributed equally to this study.

Abstract | Vegetable crops in India are facing a major threat from underground root pathogens known as root-knot nematodes. Reports suggest that India suffers 12% losses in crop yields annually due to these obligate parasites. Out of the 100 globally known *Meloidogyne* species, 14 are reported from India only. Eggplant is the major crop suffering from root-knot infestation in northern India, necessitating proper identification and management strategies. A major challenge in managing these nematodes is their proper identification of species, which is traditionally based on morpho-anatomy. These practices are laborious, time-consuming and not completely reliable, leading to poor management. However, using molecular methods can improve the accuracy of identification and minimize errors in classification. Therefore, for this study, *Meloidogyne javanica* was collected from a brinjal field and analyzed using ribosomal DNA gene sequences. The sequences obtained were then submitted to NCBI with accession no. PP711351 and further used in a phylogenetic analysis to understand their evolutionary relationship with the existing nematode database. The BLAST homology of 28S rDNA sequences showed a 99.56% sequence homology with known sequences of *Meloidogyne javanica* isolates. Moreover, the maximum-likelihood method of phylogeny indicates that *Meloidogyne javanica* PP711351 is a distinct isolate among *Meloidogyne* species. The analysis revealed the evolutionary relationships among species within the genus, providing a valuable framework for future research. Moreover, the identification of the target species, *Meloidogyne javanica* PP711351, from brinjal in this region provides significant evidence of the presence of this notorious root pathogen, and thus, proper management strategies can be developed against it.

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***Correspondence** | Faheem Ahmad, Department of Botany, Aligarh Muslim University, Aligarh-202002, India; **Email:** faheem.bt@amu.ac.in
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Introduction

Globally, plant-parasitic nematodes (PPNs) represent a significant problem for farmers, particularly in India, leading to serious crop losses. On average, nematodes reduce crop yields by about 25%, but in some fields, they can cause complete crop loss (Sasser and Carter, 1982; Seid *et al*., 2015). Among PPNs, Root-knot nematodes (*Meloidogyne* species) are a major concern to vegetable crops, affecting almost every crop and causing significant reductions in yields (Pan *et al*., 2023). The four main species of *Meloidogyne viz., M. incognita, M. javanica, M. arenaria, and M. hapla* are important due to their wide distribution and host range. Some economic crops such as tomatoes, eggplants, okra, chickpeas, cucurbit, beetroot, cowpea, Ash gourd, potato and bell peppers have experienced a reduction in yield due to this pest (Walia and Khan, 2023; Khan *et al*., 2023; Collett *et al*., 2024; Bouchtaoui *et al*., 2025). Its ability to survive in different environmental conditions makes it a serious threat in tropical and subtropical areas. The life cycle of the nematode depends on soil temperature, with the best conditions for reproduction being between 25-30°C, which is common in many farming areas. These conditions are very common in northern India, favoring *M. javanica* infestation across the region. Therefore, *M. javanica* is one of the most common and harmful *Meloidogyne* species, especially affecting the majority of economic crops in this region. This nematode penetrates through the plant roots to form root galls or root knots, which interfere with the plant's ability to absorb water and nutrients, resulting in poor growth, wilting, and lower crop yields. For example, research has shown that *M. javanica* infestations can reduce tomato yields by up to 60%, and in brinjal, the losses can be as high as 70% (Das *et al*., 2022). Nematode not only lowers the amount of the crop but also affects its quality, making the produce less appealing for sale (Jones *et al*., 2013; Sikora *et al*., 2018).

Understanding the morphological and genomic features of *M. javanica* is important for properly identifying and managing this pest. Traditionally, scientists have studied the nematode's physical traits like body shape, size, structure and perineal patterns to identify *Meloidogyne* spp. However, many species are cryptic in nature, so it can be misleading to distinguish species on the basis of morphology and therefore, more reliable approaches should be employed for reliable identification. To address these ambiguities, molecular methods have become a valuable and reliable tool. Techniques like polymerase chain reaction (PCR), DNA sequencing, and molecular markers offer more accurate and precise identification and classification of *Meloidogyne* spp. (Nisa *et al*., 2022). These methods have also assisted scientists to explore more comprehensively the genetic diversity, and evolutionary relationship of *Meloidogyne* spp. with other parasitic nematodes. Molecular methods detect variations in DNA sequences within nematode groups, with a primary focus on mitochondrial DNA (mtDNA) and nuclear ribosomal DNA (rDNA). The key genes in PPN identification include 18S, 28S, and 5.8S, as well as spacer regions such as the internal transcribed spacer (ITS), external transcribed spacer (ETS), and intergenic spacer (IGS). The large subunit rDNA (28S or LSU) contains both conserved and variable regions, such as the D2-D3 expansion segment, which is particularly useful for developing species-specific identification tests (Roberts *et al*., 2016; Cunha *et al*., 2018). Using DNA-based markers for species identification is faster, more reliable, and doesn't depend on environmental conditions or the nematode's life cycle stage (Laasli *et al*., 2025).

In this study, we employed both morphological and molecular approaches to identify *M. javanica* isolate infecting eggplant crops in northern India's Aligarh district. By combining these approaches, we aimed to develop a precision method for *M. javanica* identification so that species-specific management tools can be developed to combat this pathogen in the region.

Materials and Methods

Survey and sampling of root-knot nematode

Field surveys were carried out in 5 villages within the Aligarh district (27°88' N latitude and 78°08' E longitude). A total of five (5) eggplant roots were collected from each agricultural field. For the selection of plants, aerial symptoms like chlorosis and wilting were taken into consideration. A total of 30 plants were examined in each field to detect the existence of *Meloidogyne-induced* galls. Subsequently, root samples were obtained from the rhizosphere of the affected plants. Root samples were obtained from the rhizosphere of a plant exhibiting unfavorable symptoms of growth (Figure 1 C-D). Infected roots with characteristic galls were cleaned with tap water

to remove the soil. The females were carefully isolated from the galls by gently dissecting the infected root tissues using sterilized forceps and a fine needle under a stereomicroscope. The extracted females were then transferred to a Petri dish containing sterile water for further investigation.

Figure 1: *Pictures illustrating root-knot nematode (RKN) causing above and below-ground symptoms in eggplant: (a-b) The eggplant field showed above-ground symptoms; (c-d) The roots of the eggplant are heavily infected and show below-ground symptoms.*

Morphological identification

Root-knot nematodes were obtained from the infected eggplant (*Solanum melongena* L.) roots. Adult females were used for identification purposes for RKN species identification. A single female nematode was placed on the glass slide and cut in half just above the middle body. The anterior half was removed, and the posterior half was mounted in the cold lactophenol with 0.03% cotton blue for 24 hr at 28±2°C. The stained tissue was trimmed around the perineal pattern in a neat square using the sharp scalpel, and a coverslip was placed on the tissues. The excess liquid was absorbed using blotting paper. The glass slide containing stained tissue samples was used to see the shape and structure of the perineal pattern and to identify *Meloidogyne* species under a microscope.

To enhance the visualization of the perineal pattern, scanning electron microscopy analysis (JSM 6510LV; Jeol, Tokyo, Japan) was used. For slide preparation, ten precisely cut perineal patterns, oriented with the surface facing upwards, were placed into a small drop of 45% lactic acid on a round coverslip. A border of glyceel was applied to form a chamber around the samples. The coverslip, measuring 13 mm in diameter, was then secured to the microscope slide using glyceel along the edges. Lactic acid was subsequently removed by washing with 2% formalin. Excess solution was removed using blotting paper, and the perineal pattern was allowed to dry at room temperature (28 ± 2°C) in a glass desiccator (Isabel *et al.*, 1989). Prior to SEM the coverslip was carefully removed from the microscope slide and mounted onto the adhesive side of an SEM stub, followed by gold coating (~14 nm thickness). The resulting SEM images of the surface morphology of the perineal pattern were used for the identification of *Meloidogyne* spp. (Figure 2), following the description provided by Eisenback *et al.* (1985).

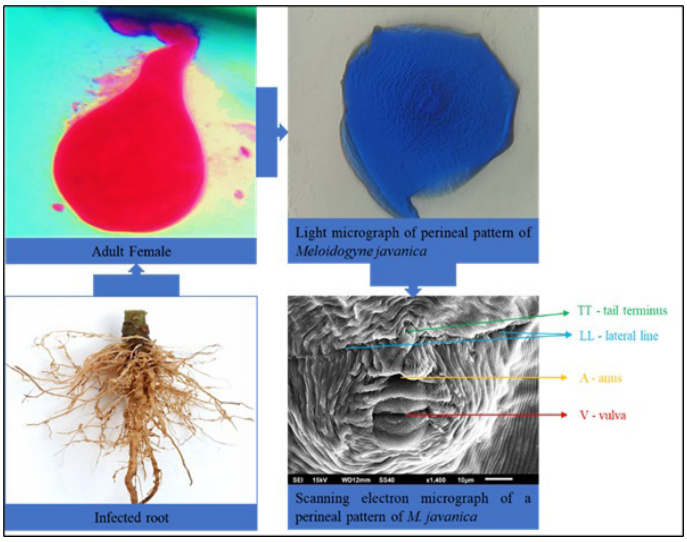

Figure 2: *Figure illustrating infected roots with RKN, M. javanica. The adult female was obtained from galls of infected root and prepared perineal pattern for microscopy. Microscopic image of the perineal pattern of M. javanica, showing a rounded to flattened dorsal arch and conspicuous lateral lines (LL) that separate the dorsal and ventral regions of the patterns. The distinct lateral line (LL), striae (S) contained the vulva (V), and anus (A) in a perineal pattern distinguishes M. javanica species from other Meloidogyne spp.*

DNA extraction, PCR and sequencing

DNA was isolated from adult female root-knot nematodes using the NucleoSpin® Tissue Kit (Macherey-Nagel). The DNA extracted from each sample was dissolved in 50 µl of BE buffer and preserved at -20°C until PCR testing was conducted. Thermal cyclers, specifically the GeneAmp PCR System 9700 from Applied Biosystems, were utilized in order to carry out the PCR amplification. The primer set used to amplify *M. javanica* included a forward primer called 28sF (ACCCGCTGAATTTAAGCAT) and a reverse primer called 28sR

(GACGATCGATTTGCACGTCA) (Folmer *et al.*, 1994). The PCR reaction mixture included 3.5µL of distilled water, 5µL of 2X Phire Master Mix, 0.25µL of Forward Primer, 0.25µL of Reverse Primer, and 1µL of DNA. The best conditions for amplification were an initial denaturation for 30 seconds at 98˚C, followed by 35 cycles of (98˚C for 5 seconds, 54˚C for 10 seconds, 72˚C for 15 seconds), and a final extension for 60 seconds at 72˚C. The PCR products were tested on 1.2% agarose gels made with 0.5X TBE buffer and 0.5 µg/ml ethidium bromide. To each 5 µl of PCR product, 1 µl of 6X loading dye was added, and the mixture was loaded onto the gel. Electrophoresis was carried out at 75V using 0.5X TBE buffer for 1-2 (hr) until the bromophenol blue dye moved close to the bottom of the gel. A 2-log DNA ladder (NEB) was used as the molecular standard. The gels were viewed using a UV transilluminator (Genei), and the image was captured under UV light with a Gel documentation system (Bio-Rad).

The PCR product, which was five microliters in volume, was mixed with two microliters of ExoSAP-IT and then incubated at 37˚C for thirty minutes. Subsequently, the enzyme was inactivated at a temperature of 80˚C for fifteen minutes. To carry out the sequencing reaction, a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) was utilized, and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) was utilized. The cleaned-up PCR products were then used to clone and sequence the DNA fragments in both directions. The quality of the sequences was checked using Sequence Scanner Software v1 (Applied Biosystems). The sequences were subsequently aligned and modified as necessary utilizing Geneious Pro v5.1 (Drummond *et al.*, 2010). A BLAST (Basic Local Alignment Search Tool) search was done at NCBI (National Center for Biotechnology Information) using the 28S rDNA gene to confirm that the sequences were from nematodes and identified them as *M. javanica*. The final consensus sequences were submitted to the GenBank database with the accession number PP711351. DNA extraction and their sequencing process are represented in (Figure 3).

Phylogenetic analysis

The phylogenetic relationship of the RKN species obtained in this study was established with known species by comparing the obtained 28S rDNA sequence with the reference sequences present in the GeneBank by using the maximum-likelihood (ML) trees through MEGA version 11 software. Bootstrap resampling was performed to assess the statistical support for the inferred relationships. The bootstrap value of 1000 was used to determine the level of support for phylogenetic branching.

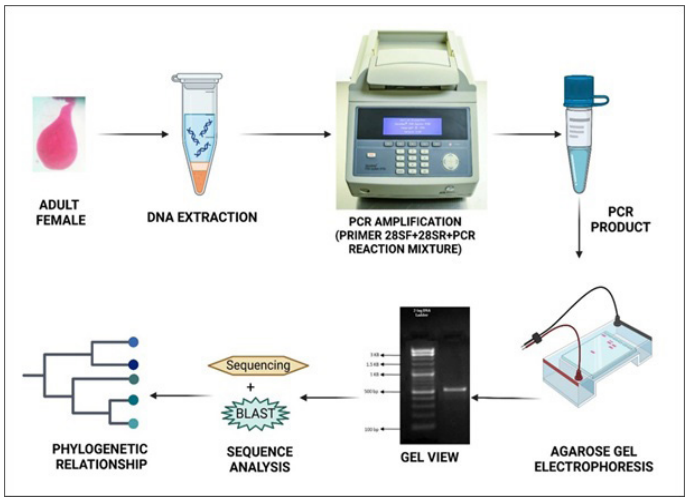

Figure 3: *This image illustrates the process of DNA analysis, starting with DNA extraction from an adult female. PCR amplification using specific primers (28SF+28SR) is followed by sequencing and BLAST analysis to determine the phylogenetic relationship, and the PCR product is visualized through agarose gel electrophoresis.*

Results and Discussion

Field symptoms and morphological assessment

The above-ground manifestations of *M. javanica* comprised irregular patches in the field, deficient plant growth, a stunted appearance, and chlorosis and wilting of the leaves. The impacted roots exhibited an enlarged, hypertrophied appearance, termed galls (Figure 1C, D). When the galled roots were dissected, Root-knot nematodes (RKN) were found. The females were pear-shaped and located inside the root's cortical layer (Figure 2A). The perineal patterns on the females were round to oval when viewed from top to bottom (Figure 2B).

Scanning Electron Microscopy images (Figure 2C) of the perineal patterns of *M. javanica* showed a moderately high dorsal arch and prominent lateral lines. This pattern was characterized by prominent lateral incisures, dividing it into dorsal and ventral areas. Several striae intersected the lateral incisures, while others arched towards the vulva. These are the main characteristic features of the RKN, *M. javanica*.

Molecular profiles and phylogenetic relationships of M. javanica

To confirm the findings of the perineal pattern, the amplification of 28S nematode gene was done and the expected band of 670 bp was observed on gel electrophoresis (Figure 4).

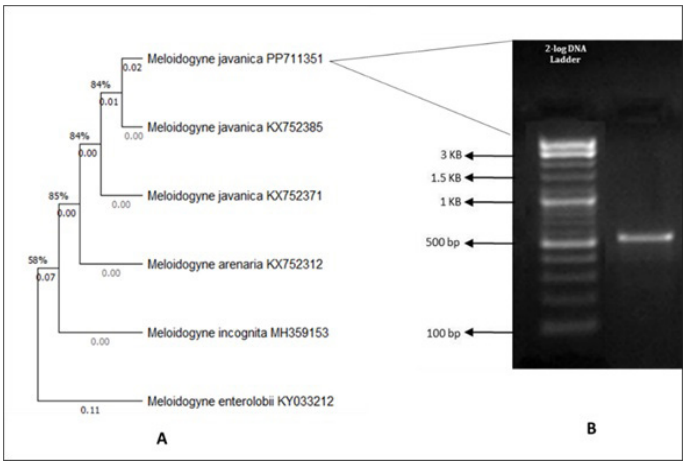

Figure 4: *(A) Maximum likelihood phylogenetic relationships of Meloidogyne javanica based on 28S rDNA; (B) PCR amplification of M. javanica with a DNA band with a length of 670 bp and DNA ladder used for estimating band sizes of PCR product visualized on agarose gels.*

The phylogenetic analysis indicated that the isolate, *M. javanica* PP711351, is closely related to other *M. javanica* isolates with minor genetic divergence as revealed by the phylogenetic tree. The high bootstrap support validates these relationships, confirming that the sequencing data accurately represents the evolutionary proximity of these species. This tree also helps to distinguish isolate *M. javanica* PP711351 from other known *Meloidogyne* species like *M. incognita* and *M. enterolobii* (Figure 4). Moreover, the tree demonstrates that *M. javanica* is a monophyletic group, meaning that all of the *M. javanica* species are more closely related to each other than to any other species. The branch separating our isolate PP711351 from known *M. javanica* isolate KX752385 has a genetic distance of 0.02, indicating slight divergence. The bootstrap values associated with these clusters are high, with 84% support, indicating confidence in the inferred relationships between these isolates. The lines connecting the species represent their genetic similarity. The shorter the line, the more closely related the species are. Therefore, in this case, the species *M. javanica* PP711351, *M. javanica* KX752385, and *M. javanica* KX752371 are very closely related, as indicated by the short lines connecting them.

Root knot disease is a serious concern to eggplant growers in Northern India (Walia and Khan, 2023). In the Aligarh district of Uttar Pradesh, various reports have suggested that there is a severe infestation of eggplant fields with *Meloidogyne* spp. (Akhter and Khan, 2018). To develop proper management strategies, identification of the pathogen is necessary. Therefore, this study investigated both morphological and molecular techniques employed to identify and characterize RKN, *M. javanica, infecting the* eggplant. In this study, *M. javanica* was detected in the samples taken from the eggplant fields of the Aligarh district through the perineal pattern. While the perineal pattern of females is helpful for distinguishing *Meloidogyne* species, this characteristic alone is no longer adequate and may lead to misidentification due to significant similarities and overlaps in perineal patterns among different species (Rusinque, 2017). Molecular techniques, such as PCR assays, are crucial for more precise identification, especially when morphological similarities exist between different nematode species (Bogale *et al.*, 2020). Molecular analysis using rDNA further confirmed the species identity, aligning with prior genetic studies. rDNA sequences, including ribosomal DNA regions such as 18S rDNA, ITS1, ITS2, and 28S rDNA, are widely used for molecular characterization. These regions show enough variation between species but are conserved enough within species to be used for phylogenetic analyses. Molecular identification using the intergenic spacer (IGS) region of ribosomal DNA has been employed in previous studies as a diagnostic marker to differentiate between various *Meloidogyne* species (Skantar *et al.*, 2023; Yang *et al.*, 2023; Carneiro *et al.*, 2024). In their 2004 study, Zijlstra *et al.* (2004) developed a PCR assay targeting the ribosomal DNA internal transcribed spacer (ITS) region to detect the cereal root-knot nematode *Meloidogyne naasi*. This method utilizes species-specific primers designed from aligned ITS sequences, allowing for accurate identification.The development of this PCR test provides a valuable tool for preventing the spread of *M. naasi*, implementing host resistance strategies, managing crops effectively, and designing appropriate crop rotation systems.

Recently, various researchers across the globe have suggested a molecular approach for the identification of *Meloidogyne* spp. The ribosomal DNA (rDNA), particularly the region (D2-D3) of the 28S rDNA, is commonly used in polymerase chain reaction (PCR)

assays to distinguish between *Meloidogyne* species. Castro-López *et al*. (2024) first reported the RKNs affecting ridge gourd in Mexico. The nematodes were identified morphologically by analyzing typical perineal patterns and confirmed through molecular methods targeting the 28S rDNA gene. This combined approach verified the presence of *M. javanica*, underscoring its impact on agriculture in the region and highlighting the importance of molecular diagnostics for accurate nematode identification and management. Hajihassani *et al*. (2023) discusses the identification and impact of *M. incognita*, on passion fruit (*Passiflora edulis*) in Florida. The study is significant as it marks the first report of this nematode infecting purple passion fruit in the United States. The species was confirmed through both morphological methods and molecular diagnostics, specifically using the D2-D3 and ITS regions of ribosomal DNA (DNA). Species-specific primers confirmed the presence of *M. incognita*.

Recent trends indicate that nematologists are turning to PCR techniques for nematode identification due to several key advantages. One of the most significant benefits is the rapid turnaround time, with results available in approximately a few hours, making it much faster than traditional methods. Additionally, PCR can handle the analysis of multiple samples simultaneously, increasing efficiency. In some cases, PCR does not require advanced nematode taxonomic expertise, reducing the need for specialized skills and saving both time and resources (Braun-Kiewnick and Kiewnick, 2018). It is recommended to use species-specific PCR for identifying tropical root-knot nematodes, as the genes of these nematodes are highly conserved, making DNA sequencing and BLAST searches alone insufficient for accurate identification (Danso *et al*., 2023). This study expands the molecular characterization of *M. javanica* and provides additional sequences that enable more precise and reliable diagnosis of the species. Moreover, the research contributes valuable insights on the widespread distribution and phylogeny of *M. javanica*. The phylogenetic analysis of the *M. javanica* isolate PP711351 revealed that it shares a close evolutionary relationship with other *M. javanica* isolates, specifically *M. javanica* isolates KX752385 and KX752371. This close relationship suggests that these isolates may have evolved from a common ancestor, indicating minimal genetic divergence among them. Furthermore, a BLAST search of the 28S rDNA sequences conducted through the NCBI database confirmed a high degree of genetic similarity between the PP711351 isolate and other known *M. javanica* isolates, with a sequence homology of 99.56%. This nearly perfect match reinforces the reliability of molecular identification methods and demonstrates genetic stability within the *M. javanica* species. Such high sequence homology is crucial for accurate species identification and understanding evolutionary relationships, which, in turn, can inform better management practices and further studies on the genetic variability within the species.

Conclusion

This study presents a comprehensive morphological and molecular characterization of RKN, *M. javanica*. By employing modern research tools such as scanning electron microscopy combined with molecular methods, this study suggests a more reliable and time-effective technique for accurately identifying root-knot nematodes, particularly *M. javanica*. Through the analysis of ribosomal DNA sequences and phylogenetic relationships, the study successfully identified the target species, *M. javanica* PP711351, and confirmed its close evolutionary link with other isolates. These findings provide valuable insights into the biology of this species and lay the groundwork for improved agricultural management strategies, minimizing the risks associated with traditional morphological identification methods.

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Novelty Statement

Morpho-anatomy and rDNA gene sequences were employed to identify *Meloidogyne* species.
A moderately high dorsal arch and lateral lines in the perineal pattern confirmed *M. javanica*.
The 28S rDNA sequences revealed a 99.56% sequence homology with *M. javanica* isolates.

Author's Contribution

Rishil Gupta: Writing-original draft, Validation, resources, methodology, formal analysis, data curation,

conceptualization.
Faheem Ahmad: Writing-review and editing, resources, conceptualization, supervision.
Khalid Z. Masoodi: Writing-review and editing, supervision.
Adnan Shakeel: Writing-review and editing.

Generative AI or AI-assisted Technology Statement
The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

References

Akhter, G. and Khan, T.A., 2018. Response of brinjal (*Solanum melongena* L.) varieties for resistance against root-knot nematode, *Meloidogyne incognita* race-1. J. Phytopharma., 7: 222-224. https://doi.org/10.31254/phyto.2018.7221

Bogale, M., Baniya, A. and DiGennaro, P., 2020. Nematode identification techniques and recent advances. Plants, 9: 1-15. https://doi.org/10.3390/plants9101260

Bouchtaoui, E.M., Smouni, A., Dababat, A.A. and Mokrini, F., 2025. Damage threshold and population dynamic of *Meloidogyne javanica* on tomato plant. J. Phytopathol., 173: 1-9. https://doi.org/10.1111/jph.70015

Braun-Kiewnick, A. and Kiewnick, S., 2018. Real-time PCR, a great tool for fast identification, sensitive detection and quantification of important plant-parasitic nematodes. Eur. J. Plant Pathol., 152: 271-283. https://doi.org/10.1007/s10658-018-1487-7

Carneiro, R.M., Souza, C.F., Mattos, V.S. and Correia, V.R., 2024. Molecular techniques for root-knot nematode identification. In: Plant-Nematode Interactions, New York, NY: Springer US. pp. 227-245. https://doi.org/10.1007/978-1-0716-3638-1_5

Castro-López, R., Gomez, G., Edeza-Urias, J.A., López-Orona, C.A., Martínez-Gallardo, J.A., Rubio-Aragón, W. and Villa-Medina, M.C., 2024. First report of *Meloidogyne javanica* parasitizing ridge gourd (*Luffa acutangula*) roots in Sinaloa, Mexico. Plant Dis., 108: 818. https://doi.org/10.1094/PDIS-06-23-1141-PDN

Collett, R.L., Rashidifard, M., Marais, M., Daneel, M. and Fourie, H., 2024. Insights into the life-cycle development of *Meloidogyne enterolobii, M. incognita and M. javanica* on tomato, soybean and maize. Eur. J. Plant Pathol., 168: 137-146. https://doi.org/10.1007/s10658-023-02741-9

Cunha, T.G.D., Visôtto, L.E., Lopes, E.A., Oliveira, C.M.G. and God, P.I.V.G., 2018. Diagnostic methods for identification of root-knot nematodes species from Brazil. Ciência Rural, 48: 1-10. https://doi.org/10.1590/0103-8478cr20170449

Danso, Y., Danso, B.A., Armooh, B. and Abugri, B., 2023. Molecular detection of root-knot nematodes (Nematoda: *Meloidogynidae*) infecting okra in Ghana. J. Agric. Food Sci. Biotech., 1: 219-223. https://doi.org/10.58985/jafsb.2023.v01i03.26

Das, S., Wadud, M.A., Chakraborty, S. and Khokon, M.A.R., 2022. Biorational management of root-knot of brinjal (*Solanum melongena* L.) caused by *Meloidogyne javanica*. Heliyon, pp. 1-9. https://doi.org/10.1016/j.heliyon.2022.e09227

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir R., Stones-Havas, S. Sturrock, S., Thierer, T. and Wilson, A., 2010. Geneious v5.4. (Web page: https://www.geneious.com) (Date accessed: March 2025).

Eisenback, J.D., Sasser, J. and Carter, C., 1985. Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). Adv. Treatise Meloidog., 1: 95-112.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol., 3: 294-299.

Hajihassani, A., Singh, P.R. and Gitonga, D., 2023. The root-knot nematode *Meloidogyne incognita* infects passion fruit in the USA. Austral. Plant Dis. Notes, 18: 1-3. https://doi.org/10.1007/s13314-023-00493-3

Isabel, M.D.O. and de A Santos, M.S.N., 1989. A technique for preparing perineal patterns of root-knot nematodes for scanning electron microscopy. J. Nematol., 21: 138-139.

Jones, J.T., Haegeman, A., Danchin, E.G., Gaur, H.S., Helder, J., Jones, M.G., Kikuchi, T.,

Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M. and Perry, R.N., 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol. Plant Pathol., 14: 946-961. https://doi.org/10.1111/mpp.12057

Khan, A., Ansari, S.A., Haris, M., Hussain, T. and Khan, A.A., 2023. *Meloidogyne* species: Threat to vegetable produce. In: Root-galling disease of vegetable plants Singapore: Springer Nature Singapore. pp. 388. https://doi.org/10.1007/978-981-99-3892-6_2

Laasli, S.E., Kallali, N.S., Legrifi, I., Kenfaoui, J., Goura, K., Mokrini, F., Barakate, M., Dababat, A.A. and Lahlali, R., 2025. Molecular diagnostics and management of phyto-parasitic nematodes. In: Molecular and biotechnological tools for plant disease management. Springer, Singapore. pp. 75-118. https://doi.org/10.1007/978-981-97-7510-1_3

Nisa, R.U., Tantray, A.Y. and Shah, A.A., 2022. Shift from morphological to recent advanced molecular approaches for the identification of nematodes. Genomics, 114: 1-13. https://doi.org/10.1016/j.ygeno.2022.110295

Pan, C., Peng, D.L.Y., Li, M., Chen, Z.J., Zhai, Y.Y., Liu, C. and Hong, B., 2023. Potential global distribution of the guava root-knot nematode *Meloidogyne enterolobii* under different climate change scenarios using MaxEnt ecological niche modeling. J. Integ. Agric., 22: 72138-72150. https://doi.org/10.1016/j.jia.2023.06.022

Roberts, D., Oliveira, C.M.G., Neilson, R. and Blok, V., 2016. Diagnose molecular de nematoides parasitos de plantas. *Oliveira, CMG*; Santos, MA; Castro, LHS Diagnose de fitonematoides. Campinas: Millennium, pp. 281-324.

Rusinque, L.C.M., 2017. Morphological Biochemical and molecular approaches to the identification of *Meloidogyne incognita meloidogyne javanica* and *Meloidogyne arenaria* in Portugal (Master's thesis, Universidade de Lisboa (Portugal)). pp. 80.

Sasser, J.N. and Carter, C.C., 1982. Root-knot nematodes (*Meloidogyne* spp.): Identification, morphological and physiological variation, host range, ecology, and control. Nematology in the Southern region of the United States. South. Cooper. Ser. Bull., 276: 21-32.

Seid, A., Fininsa, C., Mekete, T., Decraemer, W. and Wesemael, W.M., 2015. Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.). A century-old battle. Nematol., 17: 995-1009. https://doi.org/10.1163/15685411-00002935

Sikora, R.A., Coyne, D., Hallmann, J. and Timper, P., 2018. Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, pp. 876. https://doi.org/10.1079/9781786391247.0000

Skantar, A.M., Handoo, Z.A., Kantor, M.R. and Hult, M.N., 2023. First report of barley root-knot nematode, *Meloidogyne naasi* from turfgrass in Idaho, with multigene molecular characterization. J. Nematol., 55: 1-17. https://doi.org/10.2478/jofnem-2023-0051

Walia, R.K. and Khan, M.R., 2023. Root-knot Nematodes (*Meloidogyne* spp.). In: Root-Galling Dis. of Vegetable Plants, Singapore: Springer Nature Singapore. pp. 1-60. https://doi.org/10.1007/978-981-99-3892-6_1

Yang, Y., Wang, P., Yu, X., Dai, Y., Yao, H., Li, Y., Li, Q. and Hu, X., 2023. First report of *Meloidogyne javanica* infecting bletilla striata in Yunnan, China. Plant Dis., 107: 3319. https://doi.org/10.1094/PDIS-02-23-0215-PDN

Zijlstra, C., Van hoof, R. and Donkers-Venne, D., 2004. A PCR test to detect the cereal root-knot nematode *Meloidogyne naasi*. Eur. J. Plant Pathol., 110: 855-860. https://doi.org/10.1007/s10658-004-2492-6