**Molecular-genetic characterization of *Meloidogyne* (Heteroderidae) nematode species in Surkhandarya Region**

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**Abstract.** This study focuses on identifying root-knot nematodes in peanut, carrot, sugar beet, cucumber, and tomato crops in the Surkhandarya region and examining their genetic characteristics. A total of 765 root samples were analyzed using PCR and nucleotide sequencing of the 5.8S–ITS2 region of rDNA. The findings revealed minimal genetic variation within populations of *Meloidogyne javanica*, *M. incognita*, and *M. arenaria*, while interspecific differentiation was distinct. A phylogenetic tree constructed using the Maximum Likelihood method clearly separated three major clusters, reliably demonstrating the evolutionary relationships among the species. The molecular-genetic analyses highlight the high efficiency of these methods for identifying plant-parasitic nematodes and assessing their phylogenetic relationships.

**INTRODUCTION**

In recent years, the rapid growth of the world population has intensified the challenge of increasing food production efficiency and ensuring adequate food supply. From this perspective, nematodes are polyphagous plant parasites that pose a serious threat to global food security. Rising global temperatures have direct ecological and physiological impacts on the developmental dynamics of nematode populations. Elevated soil temperatures and changes in plant physiological processes accelerate nematode life cycles, increasing their reproduction rate and thereby intensifying plant infestation (Somasekhar et al., 2011; Mendy et al., 2017).

Nematodes infest various plants, and by feeding and reproducing within them, they cause a significant reduction in crop yield. In particular, root-knot nematodes of the genus *Meloidogyne* Göldi, 1892 are widespread worldwide and threaten more than 2,000 monocot and dicot plant species. Members of this genus are considered among the most destructive plant pathogens, causing nearly 12% yield loss in major crops worldwide and resulting in an estimated annual economic damage of about 100 billion USD (Cabrera et al., 2016; Bardgett, 2014; Forghani et al., 2020).

Currently, identifying the species composition of root-knot nematodes and developing effective control measures against them is of great importance (Sasser, 1980; Whitehead, 1968; Hussey, 1979; Zeng et al., 2012). In recent years, advances in molecular-genetic techniques have introduced new approaches in biological research. Notably, results obtained using mitochondrial and nuclear DNA markers allow the detection of genetic differences at both species and population levels (Bradley, 1990; Kavulukoa et al., 2016; Khanal et al., 2016; Blok et al., 2009; Ye et al., 2015). Molecular-genetic studies are especially important for species of the genus *Meloidogyne*, because although morphological variation within species is often minimal, distinct genetic differentiation has been reported (Ye et al., 2019; Hunt et al., 2009; Tigano, 2000).

Therefore, molecular-genetic analyses conducted on *Meloidogyne* nematodes are scientifically significant not only for accurately establishing their systematic classification but also for developing scientifically grounded strategies to protect agricultural crops and combat nematode infestations.

The aim of this study is to perform a molecular-genetic analysis of the species *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *M. incognita* Lordello, 1956, and *M. arenaria* Chitwood, 1952, belonging to the genus *Meloidogyne* Göldi, 1892, collected from Surkhandarya region (Uzbekistan), based on nucleotide sequencing of the ribosomal DNA 5.8S–ITS2 region.

**MATERIAL AND METHODS**

The study was conducted during the spring, summer, and autumn of 2024–2025 in farms and private household plots located in the districts of Jarqo‘rg‘on, Qumqo‘rg‘on, Sherobod, Angor, Oltinsoy, Denov, Sho‘rchi, Termiz, and the city of Termiz in Surkhandarya region, Uzbekistan. Peanut (*Arachis hypogaea*), carrot (*Daucus carota*), sugar beet (*Beta vulgaris*), and greenhouse-cultivated cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*) plants were examined. A total of 765 samples were collected from roots infested with root-knot nematodes using route and stationary sampling methods.

For morphological and molecular-genetic analyses, infested root samples were preserved in 70% ethanol. Nematodes were isolated from plant roots and rhizosphere soil using Berman’s funnel technique. Permanent slides were prepared using Seinhorst’s method. Species and genera of phytoparasitic nematodes were identified using an N-300M trinocular microscope and nematode identification keys and atlases. Temporary and permanent slides were prepared to identify species based on morphological features. Species differentiation was performed according to head and tail morphology, the structure of the esophageal region, and cuticular striation patterns (Choriyev et al., 2024; Khuramov et al., 2024).

For molecular-genetic procedures, root samples were washed with distilled water, and three adult female nematodes were isolated and placed into 1.5 ml Eppendorf tubes. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). The 5.8S–ITS2 region of ribosomal DNA, commonly used in molecular taxonomy, was amplified using the TW81 forward (5´-GTTTCCGTAGGTGAACCTGC-3´) and reverse (5´-ATATGCTTAAGTTCAGCGGGT-3´) primers (Curran et al., 1994; Kuchboev et al., 2020).

Polymerase Chain Reaction (PCR) was performed according to the following protocol: Stage 1 – DNA denaturation at 94°C for 5 minutes; Stage 2 – DNA denaturation at 95°C for 45 seconds; Stage 3 – primer annealing at 55°C for 45 seconds; Stage 4 – chain extension at 72°C for 1 minute 40 seconds; Stage 5 – final extension at 72°C for 5 minutes. Stages 2 through 4 were repeated for 35 cycles (Curran et al., 1994; Kuchboev et al., 2020).

The presence of DNA in PCR products was confirmed by electrophoresis on 1.0% agarose gel at 120 V. For DNA amplification and gel extraction, reagents produced by “Sileks M” (Moscow, Russia) were used following the manufacturer’s instructions.

DNA sequencing was carried out using the ABI PRISM® BigDye™ Terminator v3.1 kit, and the reaction products were analyzed using an ABI PRISM 3100-Avant automated sequencer (Moscow, Russia).

The obtained nucleotide sequences were analyzed using BioEdit, Clustal W, DNAstar™, and PAUP4 software.

To construct the phylogenetic tree, nucleotide sequences of *Meloidogyne incognita*, *M. arenaria*, *M. signifera*, and *M. javanica* obtained from sequencing, along with DNA sequences of *Meloidogyne* species downloaded from the National Center for Biotechnology Information (NCBI), were used. The sequences were edited in BioEdit, and consensus sequences were generated using MEGA XI software.

The phylogenetic tree for the ribosomal DNA 5.8S–ITS2 region was constructed using the Maximum Likelihood (ML) method with 1000 bootstrap replications. Additionally, the nucleotide sequence of the 5.8S–ITS2 region of *Pratylenchus penetrans* (genus *Pratylenchus* Filipjev, 1936) obtained from GenBank (NCBI) was selected as an outgroup for constructing the consensus tree.

**RESULTS AND DISCUSSION**

The results of the molecular genetic analysis revealed a 634-base nucleotide sequence of the 5.8S–ITS2 region of the rDNA (rDNA) of the Meloidogyne species *M.javanica, M.incognita,* and *M.arenaria.* It showed that there were certain differences in the nucleotide sequences between different populations of these species and samples from the NCBI database. These differences allowed us to determine the level of genetic variation between and within species, as well as to assess the degree of their phylogenetic relationship.

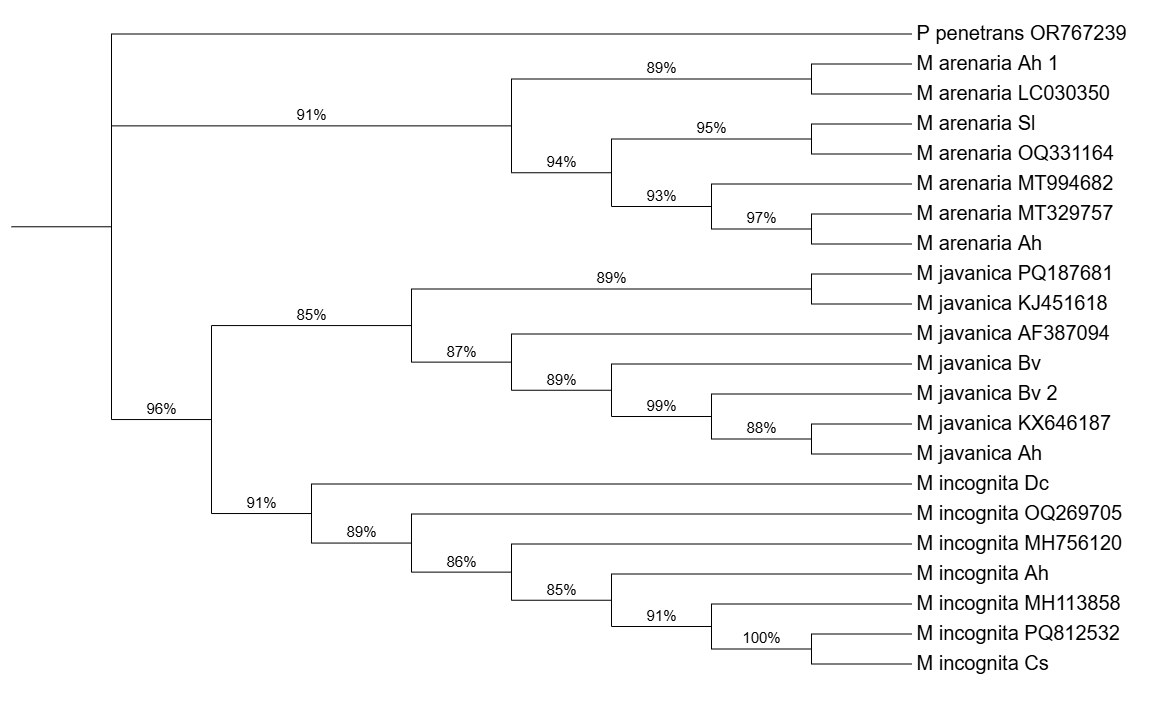
According to the analysis results, a single nucleotide difference (0.15%) was detected between *M. javanica\_Ah* and the *M. javanica\_PP756339* sequence obtained from the NCBI database, indicating a high level of genetic similarity between these samples. Likewise, *M. javanica\_Bv* differed by only 2 nucleotides (0.31%), demonstrating that intraspecific variability within this species is minimal.

When compared with other species, the *M. javanica\_Ah* sample showed 7 nucleotide differences (1.10%) with *M. incognita\_Ah*, 5 differences (0.78%) with *M. incognita\_MH113856*, and 6 differences (0.94%) with *M. incognita\_Dc*. These values indicate a relatively close phylogenetic relationship between *M. javanica* and *M. incognita*, while also confirming the presence of clear genetic divergence between the two species.

In the interspecific comparison, 32 nucleotide differences (5.04%) were identified between the *M. javanica\_Ah* sample and *M. arenaria\_Ah*, 31 differences (4.88%) with *M. arenaria\_MT994682*, and 33 differences (5.20%) with *M. arenaria\_Sl*. This high level of divergence indicates that *M. javanica* and *M. arenaria* are genetically well differentiated.

Additionally, 32 nucleotide differences (5.04%) were detected between *M. arenaria\_Ah* and *M. arenaria\_MT994682*, demonstrating the presence of considerable population-level genetic variability within the *M. arenaria* species complex.

Based on the conducted research, nucleotide sequences belonging to the rDNA 5.8S–ITS2 region were compared and analyzed. The phylogenetic analysis performed using the Maximum Likelihood method identified the genetic similarities and evolutionary relationships among *Meloidogyne* species. In constructing the phylogenetic tree, *Pratylenchus penetrans* (accession number: OR767239) was used as the outgroup. According to the results, *Meloidogyne arenaria* (Ah1, SI, Ah), *M. javanica* (Bv, Bv2), and *M. incognita* (Dc, Cs) were separated into three distinct clusters, indicating clear genetic differentiation among these species (Fig. 1).



**Fig. 1** Phylogenetic analysis of species belonging to the genus *Meloidogyne*.

The first major cluster included *M. arenaria* samples. Within this group, the phylogenetic relatedness among *M. arenaria\_Ah1*, *M. arenaria\_LC030350*, *M. arenaria\_SI*, *M. arenaria\_OQ331164*, *M. arenaria\_MT994682*, and *M. arenaria\_MT329757* was supported by bootstrap values ranging from 89% to 97%.  
These high bootstrap values serve as strong evidence confirming the genetic stability and evolutionary closeness among *M. arenaria* populations.

The second cluster grouped the *M. javanica* samples (*M. javanica\_PQ187681*, *M. javanica\_KJ451618*, *M. javanica\_AF387094*, *M. javanica\_Bv*, *M. javanica\_Bv2*, *M. javanica\_KX646187*, and *M. javanica\_Ah*). Bootstrap values within this cluster ranged from 85% to 99%, demonstrating high reliability of the phylogenetic relationships among its internal branches. Notably, the 99% bootstrap support for *M. javanica\_Bv* and *M. javanica\_Bv2* indicates that these two populations are genetically very closely related.

The third cluster included samples of *M. incognita*, combining *M. incognita\_Dc*, *M. incognita\_OQ269705*, *M. incognita\_MH756120*, *M. incognita\_Ah*, *M. incognita\_MH113858*, *M. incognita\_PQ812532*, and *M. incognita\_Cs*. Bootstrap values within this cluster ranged from 85% to 100%, indicating highly robust internal phylogenetic connections. Specifically, the 100% bootstrap support observed between *M. incognita\_PQ812532* and *M. incognita\_Cs* confirms their close genetic similarity.

Overall, the bootstrap values ranging from 85% to 100% in the phylogenetic tree indicate that the identified clusters are reliable and statistically significant. The obtained results confirm the presence of clear genetic differences among *Meloidogyne* species and demonstrate that each species represents a distinct evolutionary lineage.

The findings of this study show that root-knot nematodes of the genus *Meloidogyne* are widely distributed in the roots of peanut, carrot, sugar beet, cucumber, and tomato plants grown in various districts of the Surkhandarya region. These results align with global studies, such as those by Burrows (1988) and Cabrera (2016), which reported the widespread distribution of *Meloidogyne* nematodes in agricultural crops and highlighted genetic stability across species and populations.

Molecular-genetic analyses revealed interspecific and intraspecific variations based on nucleotide sequences of the rDNA 5.8S–ITS2 region. Our results show that genetic variation among *M. javanica* populations is minimal (0.15–0.31%), indicating a high degree of genetic homogeneity. Meanwhile, the differences observed between *M. javanica* and *M. incognita* (0.78–1.10%) indicate a close phylogenetic relationship, yet clearly distinct evolutionary lineages. The divergence values between *M. javanica* and *M. arenaria* (4.88–5.20%) confirm that they are genetically well separated, consistent with molecular differentiation previously reported by Forghani (2020) and Ye (2019).

Phylogenetic analyses revealed three major clusters: *M. arenaria*, *M. javanica*, and *M. incognita*. High bootstrap values (85–100%) within each cluster confirmed their genetic stability and phylogenetic integrity. These results demonstrate the existence of well-defined phylogenetic lineages among the species and genetic stability within populations, consistent with broader research (Burrows, 1988; Forghani, 2020).

The findings indicate that integrating morphological and molecular-genetic approaches is highly effective for identifying nematodes and detecting interspecific and intraspecific genetic variation. These data provide a reliable scientific basis for developing effective biological and agro-technological control strategies against nematodes parasitizing peanut, carrot, sugar beet, cucumber, and tomato plants (Cabrera, 2016; Ye, 2019).

**CONCLUSION**

Root-knot nematodes belonging to the genus *Meloidogyne* were found to be widely distributed in the roots of peanut, carrot, sugar beet, cucumber, and tomato plants grown in various farms and private households across the Surkhandarya region.

Molecular-genetic analyses based on the rDNA 5.8S–ITS2 nucleotide sequences confirmed distinct genetic differences among *M. javanica*, *M. incognita*, and *M. arenaria*. While genetic variation within populations was minimal, interspecific differences indicated that these species belong to separate phylogenetic clusters. The phylogenetic tree constructed using the Maximum Likelihood method clearly separated *M. arenaria*, *M. javanica*, and *M. incognita* into three main clusters. Bootstrap values ranging from 85% to 100% reliably confirmed genetic stability within species and the evolutionary relationships among them. These findings demonstrate the effectiveness of molecular-genetic approaches in identifying nematode species and understanding their phylogenetic relationships.

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