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MOLECULAR IDENTIFICATION OF *MELOIDOGYNE INCOGNITA* INFECTING *BASELLA ALBA* IN BANGLADESH

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Abstract

Indian spinach (*Basella alba*) plants were found to be infected with root-knot nematode in a pot experiment of Plant Pathology division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The molecular diagnosis of root-knot nematodes of Indian spinach at species level was done. Utilizing DNA sequencing of the rDNA 28S D2/D3 gene and PCR with SCAR primers *Meloidogyne incognita* was detected from infected Indian spinach.

Keywords: Indian spinach, *Meloidogyne incognita*, SCAR primer, PCR amplification, Bangladesh.

Indian spinach (*Basella alba* L.) is a popular green leafy vegetable grows well throughout Bangladesh. This vegetable is very popular and rich in vitamin A, vitamin C, calcium and iron. During the year 2022-23, about 1,07955 metric tons of Indian spinach produced in 28,364 acres of land in Bangladesh (BBS, 2023). The yield of Indian spinach is reduced due to infestation of various diseases such as leaf spot, foot rot, anthracnose, stem rot and root knot (Talukder, 1974). In Bangladesh, four species of root-knot nematode (RKN) association is common in many crops. They are *Meloidogyne incognita, M. javanica, M. graminicola* and *M. arenaria*. Among them, *Meloidogyne incognita* is the most abundant (Mian, 1986). According to Castillo and Jiménez-Díaz (2003), *M. incognita* and *M. javanica* are the most common and damaging nematode species infecting Indian spinach. In Bangladesh, previously root-knot nematodes species have been identified from brinjal and tomato based on molecular techniques (Das *et al.*, 2021; Elahi *et al.,* 2024). However, the molecular identification of *Meloidogyne* spp. of Indian spinach in Bangladesh has not been done so far. Hence, the present study was carried out to identify root-knot nematode species of Indian spinach based on molecular techniques.

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Fig. 1. A. Root-knot nematode infected Indian spinach plot B. Severely infected Indian spinach root C. Perineal pattern of *M. incognita* obtained from Indian spinach roots.

Seeds of Indian spice (var. BARI Puishak-1) were sown in the pot house of Plant Pathology division (PPD), Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh (23° 59′ 20.4504" N, 90° 25′ 5.4012" E) in November 2022. At 45 DAS, it was noticed that the Indian spinach plants with the symptoms of pale yellow leaves, declined plants with big root galls. These symptoms are typical symptoms of root-knot nematode infestation (Fig. 1). The collected samples were brought to the laboratory of PPD and stored at 4 °C for further use. For perineal patterns preparation, female nematodes were dissected under compound microscope following the protocol of (Sasser *et al.*, 1983). A dissecting needle was used to remove the posterior part of female nematode and the cuticle was trimmed in a square pattern in the center. The body was transferred in a glycerol solution. This process was done for up to ten patterns for the same *Meloidogyne incognita* species. Finally, the slide was covered with cover slip and sealed with nail polish. The perineal region generally had an angularly oval structure with a high dorsal arch. Inverted-V shape was formed by striae in the dorsal to the tail. Striae were in distinct waves which bent towards the lateral lines. Striae were straighter with an oval appearance in ventral region (Jepson, 1987). Nematode galls were collected from the infected m. spinach plants roots and second stage juveniles were collected from the rhizosphere soil through Baermann funnel method as source of DNA. Genomic DNA was extracted using Wizard DNA purification kit (Promega Corporation, Madison, WI, USA) following the standard protocol from eggs of the galls and second stage of juveniles (approx. 150), which were counted under stereo-microscope. The DNA quality was measured using spectrophotometer at the absorbance ratio A260/280. The quantity was 1.89 which is considered as pure DNA. For molecular identification, SCAR primer pair;

Inc-K14- F/Inc-K14-R (CCCGCTACACCCTCAACTTC/ GGGATGTGTAAATGCTCCTG) and a universal primer pair; RK28SF/R (CGGATAGAGTCGGCGTATC/GATGGTTC GATTAGTCTTTCGCC) was

used as universal primer pair for the targeted gene 28S D2/D3 (Ye *et al*., 2015). PCR were performed in a thermal cycler (Bio-Rad PTC-200, California, USA) in a total volume of 25 µL for both type of PCR. Per PCR reaction containing 12.5 µL GoTaq Green master mix (Promega Corporation, Madison, WI), 9.5 µL nuclease-free water, 1 µL of each primer (10 µM), and 1 µL template DNA. The PCR condition for universal primer (genus specific) RK28SF/R was: one cycle of denaturation at 94 °C for 4 min, followed by 35 cycles at 94°C for 30 s, 53 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 8 min. Moreover, the PCR condition for *M. inocgnita* specific primers (SCAR) was: one cycle of denaturation at 95 °C for 5 min, followed by 40 cycles at 94°C for 30 s, 55 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The genus-specific marker RK28SF/R identified *Meloidogyne* genus, which produced the amplicon size of 612 bp. The amplified PCR product was sequenced (National Institute of Biotechnology, Bangladesh) and submitted to GenBank under accession no. OQ207705. Blast analysis of the obtained sequence of the isolate found 97% nucleotide homology with *M. incognita* isolates MK102788, MK102791, and MK102789 that were previously identified in USA and submitted in GenBank database (National Center for Biotechnology Information [NCBI]) under the US National Institute of Health, Bethesda, MD, USA.

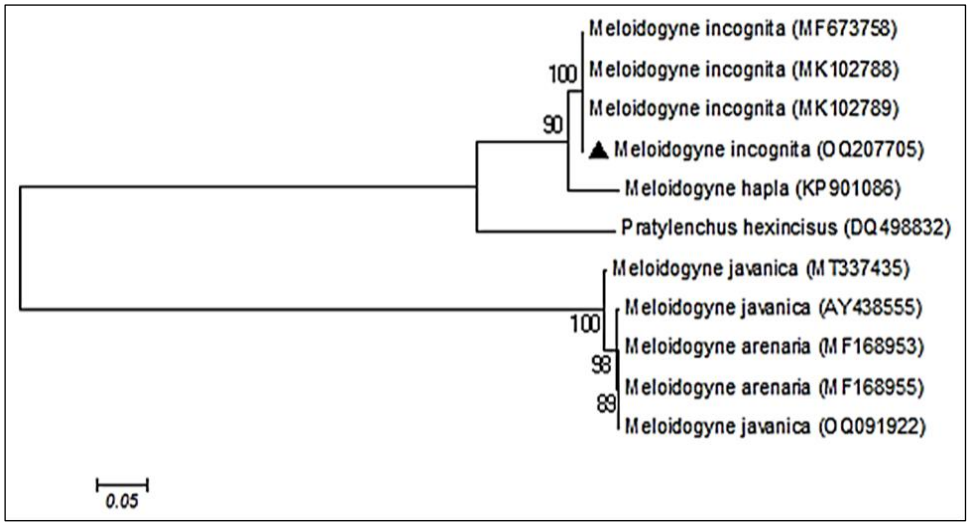

Fig. 2. Phylogenetic relationship of *M. incognita* along with other *Meloidogyne* spp. concluded by Tamura-Nei model of the 28S gene sequences using MEGA 6.0 software. The isolate used in this study was highlighted in tri-angle and *Pratylenchus hexincisus* (DQ498832) was used as out group. Values at the nodes indicate bootstrap support values based on 100 replicates. GenBank accession numbers are indicated in parentheses.

Phylogenetic analysis of the 28S gene sequence data was done by means of Maximum Composite Likelihood (MCL) method using MEGA 6.0 software. The sequence distance was calculated by Tamura-Nei parameter model. The sequence alignments of the rDNA regions were performed utilizing ClustalW program. Bootstrap values were obtained 1000 replicates to determine the support from each group. In the Phylogenetic tree, the isolate of Bangladesh (OQ207705) was placed in distinct *M. incognita* group with 100% bootstrap support while other species of *Meloidogyne* clustered in different group (Fig. 2). Moreover, SCAR primer pair produced a specific fragment of 399 bp for *M. incognita* (Fig. 3). According to Tesarova *et al*. (2003), utilization of DNA sequencing and species-specific methods are better options for *Meloidogyne* spp. diagnosis compared to conserved ITS regions or morphological identification. In addition, for the identification of tropical root-knot nematode, species-specific PCR recommended because of the genes of tropical root-knot nematodes are too conserved to identify only with DNA sequencing method and BLAST search (Danso *et al*., 2023).

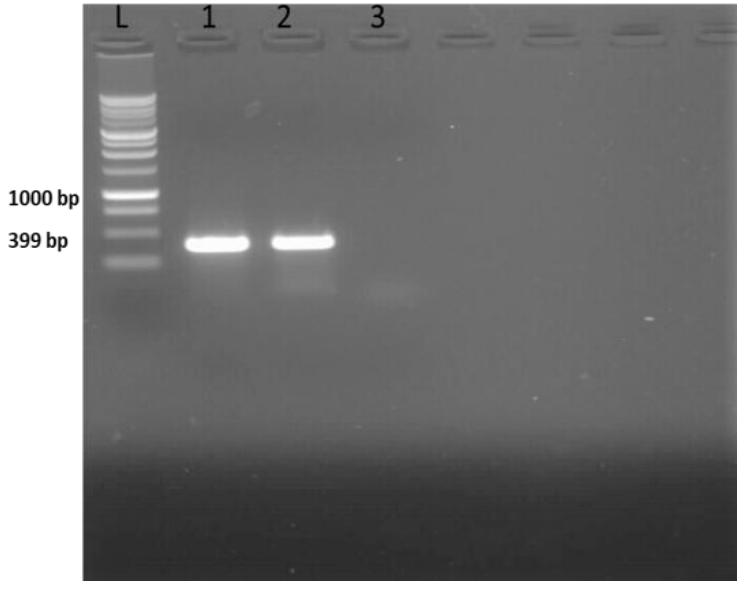

Fig. 3. Amplified product (399 bp) with *Meloidogyne incognita* species–specific primer pair Inc-K14-F/Inc-K14-R. L denotes 1 kb-DNA ladder; Lane 1 was positive control (GenBank acc.-OR351387); Lane 2 was Indian spinach isolate (OQ207705) and Lane 3 was nuclease-free water (negative control).

In Bangladesh, for the first time, *M. incognita* the root-knot nematode, infecting Indian spinach was identified by molecular markers. The results showed that *M. incognita* was present in Indian spinach in the Gazipur district of Bangladesh.

References

BBS (Bangladesh Bureau of Statistics), (2023). Year Book of Agricultural Statistics of Bangladesh. Bangladesh Bureau of Statistics, Ministry of Planning, GOB. Dhaka, Bangladesh, p: 278.

Castillo, P. and R. M. Jiménez-Díaz. 2003. First report of *Meloidogyne incognita* infecting spinach in Southern Spain. *Phytopatholgy.* DOI: https :// doi. org/ 10.1094 /PDIS. 2003.87. 7.756.

Danso, Yaw., B. A. Danso., B. Armooh and B. Abugri. 2023. Molecular detection of root-knot nematodes (Nematoda: Meloidogynidae) infecting okra in Ghana. *J. Agri. Food Sci. Biotech.* **1**(3): 219-223.

Das, S., M. A. Wadud and M. A. R. Khokon. 2021. Genetic variation of *Meloidogyne* spp. of brinjal reveals their difference in pathogenicity and hatching. *Arch. Phytopathol. Plant Protect.* **54**:19-20, 2375-2396. DOI: 10.1080/03235408.2021.1983383.

Elahi, F., M. M. Islam., M. I. Faruk and M. M. Rahman. 2024. Molecular identification of root-knot nematodes (*Meloidogyne* spp.) of tomato at Gazipur in Bangladesh. *Bangladesh J. Agri*. **49**(1): 136-140. DOI: https://doi.org/10.3329/bjagri.v49i1.74044.

Jepson, S. B. 1987. Identification of root-knot Nematodes *Meloidogyne* species. CABI Wallingford, UK. p. 265.

Mian, I. H. 1986. Plant parasitic nematodes associated with some crop species in Bangladesh. *Bangladesh J. Plant Pathol.* 2(1):7–13.

Sasser, J. N., J. D. Eisenback, C. C. Carter and A. C. Triantaphyllou. 1983. The international *Meloidogyne* project- its goal and accomplishments. *Annual Review of Phytopathology* **21**(1): 271-288.

Talukder, M. J. 1974. Plant diseases of Bangladesh. *Bangladesh J. Agril. Res.* **1**: 61-86.

Tesarova, B., M. Zouha and P. Rysanek. 2003. Development of PCR for specific determination of root-knot nematode *Meloidogyne incognita*. Plant Protec. Sci. **39**(1): 23-28. DOI: https://doi.org/10.1094/PDIS09-18-1539-RE.