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Short Communication

MOLECULAR IDENTIFICATION OF ROOT-KNOT NEMATODES (*Meloidogyne* spp.) OF TOMATO AT GAZIPUR DISTRICT IN BANGLADESH

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Abstract

Four root-knot nematode infected tomato root samples were collected from different locations of Gazipur, Bangladesh. Two methods of PCR were used to detect *Meloidogyne* spp. in tomato roots. One is molecular identification by DNA sequencing of the ribosomal DNA (rDNA) 28SD2/D3 gene, and another is PCR using species-specific SCAR primers. In the phylogenetic tree, the obtained sequences; OR351387, OR351388, OR351389, and OR351390 clustered with specific *Meloidogyne incognita* clade. Species-specific primers produced a fragment of 399 bp for *Meloidogyne incognita.* Thus, utilizing species-specific markers and analyzing the results of a phylogenetic tree made from the amplified 28S D2/D3 gene region revealed the association of *Meloidogyne incognita* on tomato root-knot disease in Bangladesh.

Keywords: *Meloidogyne incognita*, Root-knot disease, 28SD2/D3 gene, SCAR primers

Introduction

Meloidogyne spp. are highly damaging and yield reducing plant-parasitic nematode of tomato (*Solanum lycopersicum* L.) production. The average loss of crop due to Root-knot nematode (RKN) infestation could be reach up to 15% (Timm and Ameen, 1960). The species which is responsible for the gall formation on majority of crop roots are *M. incognita, M. arenarea*, *M. hapla* and *M. javanica* (Moens *et al.*, 2009). However, the species of *Meloidogyne* that causes root-knot of tomato has not been properly investigated in Bangladesh. It is assumed that *M. incognita* causes root-knot disease of tomato. For the proper identification of RKN species of tomato, molecular tools have been progressively developed in past 20 years. PCR and DNA sequencing methods are very fast, sensitive, and applicable for any stage of nematodes population (Ye *et al.*, 2019). So, the current study was proposed to identify the causal agent of root-knot of tomato utilizing PCR and DNA sequencing.

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Materials and Methods

In October-December 2022, four RKN infected tomato root samples were collected from Kapashia, Dhirasrom and BARI campus of Gazipur. The samples showed typical symptoms; stunted plants, pale yellow leaves and big galls on the roots (Yigezu Wendimu, 2021) (Fig.1.A-B). For molecular identification, SCAR primer pair; Inc-K14-F/Inc-K14-R (CCCGCTACACCCTCAACTTC/GGGATGTGTAAATGCTCCTG) and a universal primer pair; RK28SF/R (CGGATAGAGTCGGCGTATC/GATGGTTC GATTAGTCTTTCGCC) were used to target gene 28S D2/D3. DNA was isolated from approx. 150 second stage juveniles (J2) (Figure 1.C) and was extracted following Wizard genomic DNA purification kit (Promega Corporation, Madison, WI, USA). DNA quality was measured using spectrophotometer at A260/280 (1.91). PCR were performed in a thermal cycler and per PCR reaction containing 12.5 µL GoTaq Green master mix, 9.5 µL nuclease-free water, 1 µL of each primer (10 µM), and 1µL template DNA. The PCR condition for universal primer 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 (Manojkumar and Somashekharappa, 2022). Moreover, the PCR condition for *M. incognita* 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 (Randig *et al.*, 2022).

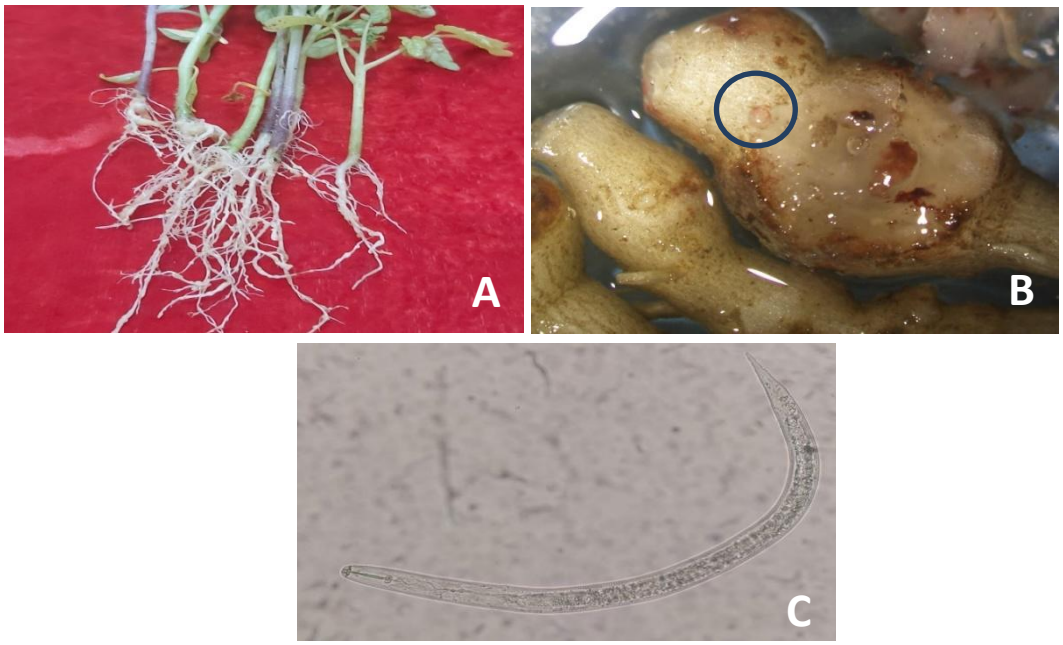

Fig. 1. (A). Root-knot nematode infected tomato roots (B). Female eggs inside the roots (C). Second satge juvenile of *Meloidogyne* spp.

Results and Discussion

SCAR primer pair produced a specific fragment of 399 bp for *M. incognita* (Fig. 2.). RK28SF/R primer pair amplified PCR 612 bp products for the isolates and the purified DNA was successfully sequenced (National Institute of Biotechnology, Bangladesh) and deposited to GenBank. The possible identities of the isolates were established by comparing sequences with those in Gen Bank database (National Center for Biotechnology Information [NCBI]) under US National Institute of Health, Bethesda, MD, USA. Blast analysis of the obtained sequences of the isolates were found 97-99% nucleotide homology with different *M. incognita* isolates that were previously submitted in GenBank database (NCBI), MD, USA (Table 1). Phylogenetic analysis of the 28S gene sequence data was done by means of Maximum Composite Likelihood method using MEGA 10.0 software. The sequence distance was calculated by Tamura and Nei, 1993; parameter model (Tamura and Nei, 1993). Bootstrap values were obtained 500 replicates to determine the support from each group. In the Phylogenetic tree, the isolates of Bangladesh; OR351387, OR351388, OR351389 and OR351390 were placed in distinct *M. incognita* group (Fig. 3.). Traditionally, morphology-based identification such as body length, morphology of sexual organs, mouth and tail parts provide inadequate information. Previously in Bangladesh, root-knot nematodes species have been identified from different vegetables and fruit crops based on only morphological characters (Elahi *et al.*, 2021).

Table 1. Blast results of isolates after sequencing and their highest homology with gene bank strains

Isolates	BLAST result	BLAST homology	Query cover	Type strains
OR351387	*Meloidogyne incognita*	99.13%	100%	OM522593
OR351388	*Meloidogyne incognita*	99.13%	100%	MF177881
OR351389	*Meloidogyne incognita*	97.92%	100%	MF177882
OR351390	*Meloidogyne incognita*	98.72%	100%	MF177880

However, this method cannot provide clear variation among closely related taxa and also for this work, required highly skilled taxonomist (Oliveira *et al.*, 2011). On the other hand, there are several advantages of PCR method in molecular diagnosis of *Meloidogyne* spp. Utilizing DNA sequencing and species-specific PCR for the detection of nematodes are the better options compared to conserved ITS regions or morphological identification (Tesarova *et al.*, 2003). Moreover, for the identification of tropical root-knot nematode, species-specific PCR is 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).

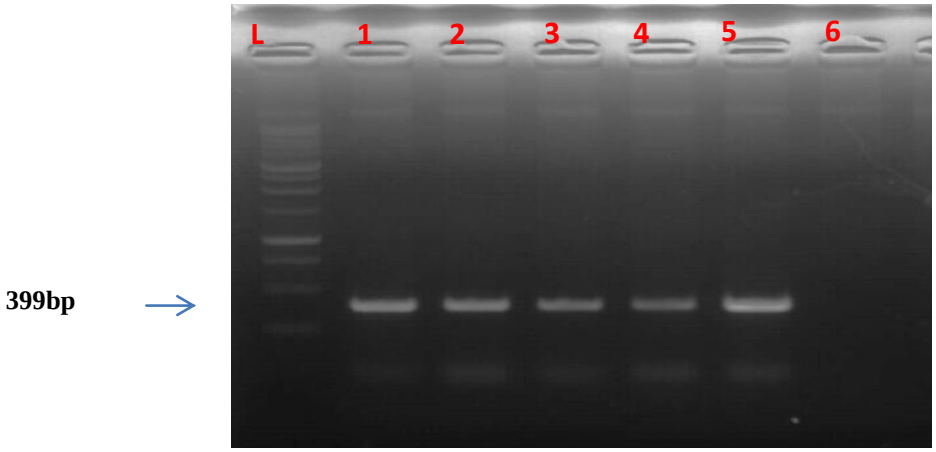

Fig. 2. Specificity of Inc-K14-F/Inc-K14-R-based PCR assay for the detection of *Meloidogyne incognita* in symptomatic tomato plants. L denotes 1 kb ladder, 1= positive control, 3-5= samples, 6= negative control.

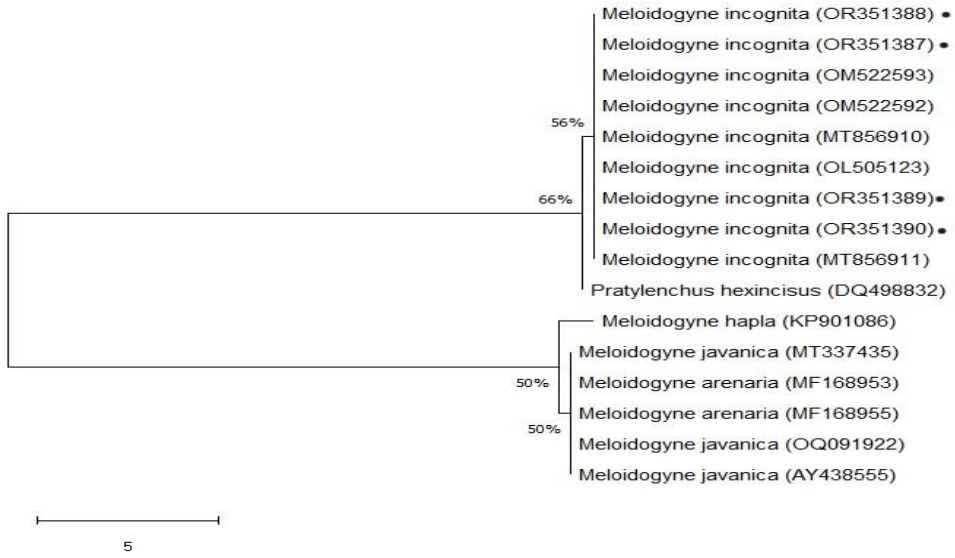

Fig. 3. Phylogenetic relationship of root-knot nematodes infecting tomato concluded by Tamura-Nei model of the 28S gene sequences. *Pratylenchus hexincisus* (DQ498832) was used as the out-group. Bootstrap support values for maximum likelihood (ML) greater than 50% are given at the nodes. Isolates obtained in this study are indicated with black dot.

Conclusion

It is the first report of molecular characterization for root-knot nematodes of tomato in Bangladesh. The results showed that *M. incognita* was present in several tomato-growing fields in the Gazipur district of Bangladesh.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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