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Morphological and Molecular Characterization of Root Knot nematode (*Meloidogyne* spp.) in Carrot (*Daucus carota* L.) from Tamil Nadu, India

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ABSTRACT: Root malformation is the major hinder faced by carrot growers. Root knot nematodes, *Meloiodogyne* spp. acts as a prime cause for the carrot root malformation. Hence, an extensive random survey was conducted in different locations from leading carrot growing districts of Tamil Nadu *viz.*, The Nilgiris, Dindigul, Erode and Krishnagiri districts for root knot nematodes parasitizing carrot, and their infestation was recorded everywhere except Erode. The compound microscopic images of posterior cuticular pattern of females collected from tropical and temperate ecosystems revealed the high dorsal arch and flattened ovoid shape, vindicating *Meloidogyne incognita* and *M. hapla,* respectively. The 560 bp amplicon 18S rDNA region of females from the tropical and temperate ecosystems of Tamil Nadu were greater than 98 per cent similar to *M. incognita* and *M. hapla,* respectively. The present study confirmed the parasitisation of *M. incognita* and *M. hapla* in carrots cultivated from tropical and temperate ecosystems of Tamil Nadu, respectively.

Keywords: Carrot, root knot nematodes, posterior cuticular pattern, polymerase chain reaction and Tamil Nadu.

INTRODUCTION

Carrot (*Daucus carota* L.) is one of the most widely cultivated root vegetables in the Apiaceae family. Because of its high content of carotenoids, anthocyanins, dietary fibre, vitamins and other nutrients, their storage root is extensively used. Salad, pickle, halwa and juice are just a few of the cuisines made from carrot (Potter *et al*., 2011). Various genera of plant parasitic nematodes have been recorded from carrots grown across several continents. Nonetheless, root knot nematodes, *Meloidogyne* spp. remains a severe menace in the carrot ecosystem around the world, as it has the capability of causing 100% yield loss (Davis & Nu, 2007). *M. incognita, M. javanica, M. arenaria, M. hapla, M. polycephannulata, M. brevidens, M. fallax, M. chitwoodi* were the major species recorded from the carrot growing regions globally (Charchar *et al*., 2009; Cunha *et al*., 2021; Hay and Pethybridge, 2005; Medina-Canales *et al*., 2019; Wesemael & Moens, 2008)

Though carrot cultivation has commenced in hill stations of Tamil Nadu a centennial ago, it has come to the limelight as one of the prime crops amidst the plains recently. The carrot growers from both ecosystems of Tamil Nadu were struggling with severe marketable yield loss due to carrot root malformation. Root knot nematodes act as one of the prime causes for root malformation (Anita & Selvaraj, 2011).

Species characterization of *Meloidogyne* is foremost to device an ecofriendly and effective management practice against root knot nematodes (Cenis, 1993). However, the earlier surveys in carrots from the temperate regions of Tamil Nadu were concluded based on conventional morphometrics only, whereas no attempt was made yet for species characterization of root knot nematodes infesting carrot in the tropical regions. In the present study, we have endeavoured the species identification of root knot nematodes infesting carrot using both traditional morphological method and polymerase chain reaction (PCR) based molecular method.

MATERIALS AND METHODS

A. Study area

The survey was conducted in different locations from major carrot growing districts of Tamil Nadu *viz.*, The Nilgiris, Dindigul, Erode and Krishnagiri districts for root knot nematodes infesting carrot prior to harvest in 2020 (Fig. 1). Soil and root samples were collected from the infested fields with GPS details (Barker & Campbell, 1981).

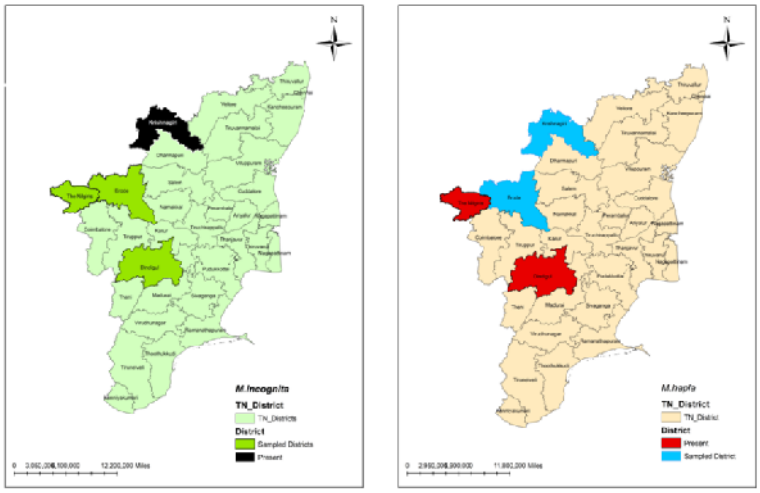


Fig. 1. Distribution of *Meloidogyne* spp. in carrot from tropical and temperate regions of Tamil Nadu.

B. Morphological characterization of root knot nematodes

The adult root knot nematode female **(**Fig. 2) was extracted from the roots using the Acid Fuchsin – Lactophenol method (Bridge & Page, 1982). The posterior cuticular pattern was trimmed from the adult female and mounted on Anhydrous Glycerine for identification (Eisenback *et al.*, 1980).

Fig. 2. Root knot nematode - Adult female.

Fig. 3. Carrot root malformation due to root knot nematode.

C. Molecular characterization of root knot nematodes

A sterilized gravid root knot nematode female was crushed and transferred to a microfuge tube containing worm lysis buffer (0.2 M NaCl + 0.2 M Tris base + 1.5 µl beta Mercaptoethanol + 1 µl Proteinase K). The microfuge tube with the solution was mini spun and dry bathed at 60°C for 3 hrs followed by 99°C for 10 mins in the thermal gradient cycler. The DNA was stored at – 20°C until use (Holterman *et al*., 2006).

Polymerase Chain Reaction (PCR) was carried out in the thermal gradient cycler (Bio Rad T 100) with microfuge tube having 10 µl volume (5µl 2X master mix, 1 µl each forward and reverse primers, 1 µl double sterile distilled water, and 2 µl DNA template). The cosmo oligos used in our characterization of 18s rDNA was designed based on the primer sequence from Joyce *et al*. (1994), and the sequences were TW 81- F (5' GTTTCCGTAGGTGAACTGC 3') and AB 28 – R – (3' ATATGCTTAAGTTCAGCGGGT 5'). The thermal cycler programming conditions for PCR were as follows: Initial denaturation at 95°C for 5 mins, followed by 40 cycles of denaturation at 95°C for 45 secs, annealing at 56°C for 45 secs, extension at 72°C for 90 secs and termination with a final extension at 72°C for 10 mins. The PCR amplicons after one per cent Agarose gel electrophoresis at 60 volts for 60 mins were documented in a Gelstan 4X advanced gel documentation system with Mediccare software.

The PCR products were purified based on the manufacturer's protocol (NucleoSpin[@] Gel and PCR Cleanup) and sequenced at Eurofins Genomics India Pvt Ltd, Bengaluru. The DNA sequences were locally aligned with previously reported sequences of root knot nematode species in the NCBI using the BLAST tool (Altschul *et al*., 1990). The resulting sequence from

CAP3 was used as a combined sequence for submission into NCBI (Huang & Madan, 1999).

D. Phylogenetic analysis

A phylogenetic tree was constructed using MEGA X (Kumar *et al*., 2018) for the 18S rDNA sequences obtained in our study, and the phylogeny was evaluated using neighbour joining (NJ) method by 1,000 bootstrap replications with a minimum cut off value of 70 per cent.

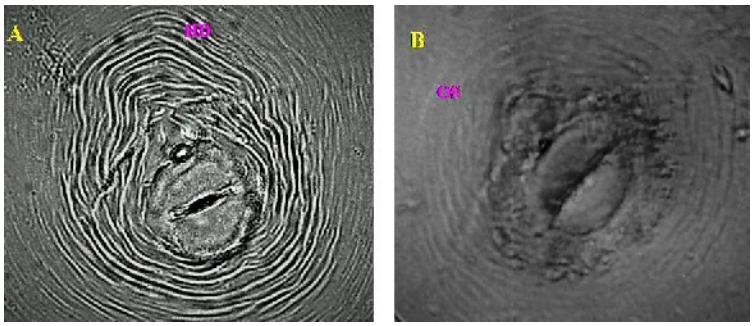
RESULTS AND DISCUSSION

A. Distribution of Meloidogyne spp. in carrot growing regions of Tamil Nadu

The present field survey conducted in 36 different locations from four leading carrot-producing districts of Tamil Nadu indicated the presence of root knot nematodes in eight locations except Erode. The infested carrot roots exhibited various forms of malformation *viz.*, forking, galling and bearding (Fig. 3). The root knot nematodes disrupt the growing root tip of carrot roots to become forked, distorted, ramified, galled and stunted (Ralmi *et al*., 2016; Seo *et al*., 2015).

B. Morphological characterization of Meloiodgyne spp. in carrot growing regions of Tamil Nadu

The posterior cuticular pattern of the adult female from the carrot grown in tropical regions has a high dorsal arch with coarse, closely placed zig zag striae and a distinct whorl in the tail terminus, whereas the perineal pattern of females from the carrot grown in temperate regions was flattened ovoid or roughly circular with very fine striae and subcuticular punctations between the anus and tail terminus. The punctations were not spotted in the former, while distinct whorl was not observed in the latter. The lateral ridges were absent in both species (Fig. 4). The posterior cuticular patterns of females collected from the tropical and temperate ecosystem of Tamil Nadu were very similar to *M. incognita* and *M. hapla*, respectively as mentioned by Eisenback *et al.* (1980). Identification of *Meloidogyne* spp. through visual aspect and shape of whole perineal pattern and arches, striations, punctuations, lateral lines and phasmids in the posterior cuticle of the matured adult female are widely followed conventional taxonomic method from Chitwood (1949) to till date. Diagnosis of *Meloidogyne* spp. through analysis of perineal pattern is an economic one as it is less expensive and easier (Seesao *et al*., 2017).

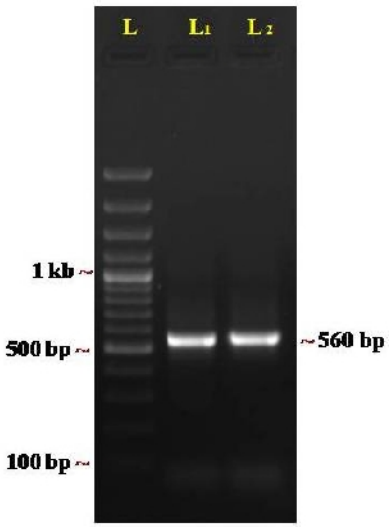


(HD – High dorsal arch, OS – Ovoid and flattened in shape)

Fig. 4. Posterior cuticular pattern of adult females (a) *M. incognita* (b) *M. hapla***.**

C. Molecular characterization of Meloiodgyne spp. in carrot growing regions of Tamil Nadu

The 560 bp amplicon of 18S rDNA region of the adult females from tropical regions (Accession Number: MZ965096) and temperate regions (Accession number: MZ964891) of Tamil Nadu were greater than 98 per cent similar to sequences of *M. incognita* and *M. hapla* available in the NCBI GenBank (Fig. 5). The size of amplicon of female's 18s rDNA was alike to Arun *et al.,* (2019) as 560 bp. Morphological characterization of root knot nematode species is time consuming and highly skilled technicians oriented (Blok *et al*., 2002). Classical morphological taxonomy of *Meloidogyne* spp. mainly depends on the posterior cuticular patterns of an adult female. Posterior cuticular patterns of many common tropical species like *M. incognita* resemble emerging tropical species like *M. enterolobii* and perineal of *M. hapla* has a close resemblance to *M. exigua* in their appearance leading to confusion (Conceição *et al*., 2012). Molecular confirmation of *Meloidogyne* spp. based on rDNA sequences brings forth the precise identification of species. The variety and stability in the multicopy base of the rDNA is a vantage for the discrimination of the major root knot nematode species (Skantar *et al*., 2008).



(L – 100 bp ladder, L_1 – *M. incognita*, L_2 – *M. hapla*)

Fig. 5. PCR products of 18s rDNA of *Meloidogyne* spp. resolved in 1% agarose gel.

D. Phylogenetic relationships of 18S rDNA of Meloidogyne spp. in carrot from Tamil Nadu

The phylogenetic tree was rooted with an outgroup as *M. enterolobii*. The phylogenetic tree was divided into two main clades, *i.e.*, one as *M. hapla* and one as *M. incognita*, apart from an outgroup. In the present study, the phylogenetic relationships were concurred extensively, *i.e.*, the DNA sequences were separated into two clades as logically anticipated without any discrepancy (Fig. 6). The rDNA region genes like 18S, 28S, 5.8S and the mtDNA region genes like cytochrome C oxidase subunits I and II are the main targets in analysing the phylogenetic relationship of root knot nematodes (Pereira *et al*., 2016).

The species of root knot nematode vandalizing carrots from tropical ecosystems of Tamil Nadu was concluded as *M. incognita* using perineal pattern diagnosis and 18S rDNA sequencing. Formerly, Nisha *et al.*, (2012) reported the residence of *M. incognita* in carrots from the plains of Kerala. Likewise, *M. incognita* was found to spread across continents *viz.*, Brazil (Cunha *et al.*, 2021), United States (Ye *et al.*, 2019), Australia (Hay & Pethybridge, 2005), Europe (Greco *et al.*, 2018) in carrot growing ecosystems. Investigations on the *Meloidogyne* spp. in different continents and at different years manifested *M. incognita* as the most encountered damaging species in the tropics (Kayani *et al*., 2013).

M. hapla was confirmed as the root knot nematode species mutilating carrots grown under temperate ecosystems of Tamil Nadu based on morphological and molecular characterization. Earlier, Seenivasan (2017); Sowmya *et al*., (2018) indicated t he habitancy of *M. hapla* in carrots from the Kodaikanal and the Nilgris, respectively. Similarly, *M. hapla* was found to be infective in the carrot across the continents, *viz.*, Brazil (Cunha *et al.*, 2021), Czech Republic (Douda *et al.*, 2021), Australia (Hay and Pethybridge, 2005), United States (Mitkowski *et al.*, 2002). *M. hapla* is a serious pest of the temperate ecosystem around the world with more than 550 host crops and weeds, whereas it is less infective on cereals or grasses (Ye *et al*., 2019).

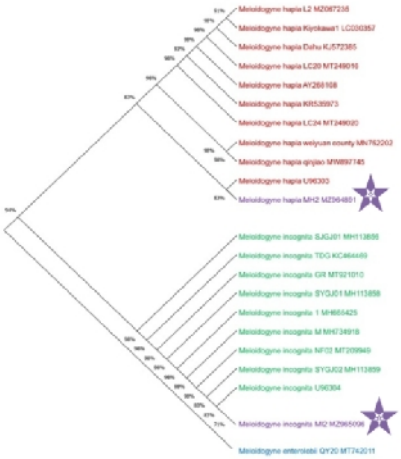


Fig. 6. Phylogenetic relationships of 18S rDNA of *Meloidogyne* spp. infesting carrot from Tamil Nadu.

CONCLUSION

The present study substantiated the parasitisation of *M. incognita* and *M. hapla* from carrots grown in tropical and temperate regions of Tamil Nadu, respectively based on morphological and molecular characterization. In the future, species confirmation of root knot nematodes can be attempted using species specific SCAR primers and Loop mediated isothermal amplification for more precise and rapid detection. Based on this information on species characterization, the best management strategy suited for each species of root knot nematode can be designed to produce blemish free carrots.

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Conflict of Interest. The results furnished in this paper were from our own research and there were no conflicts from other research scholars or scientists.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of molecular Biology*, *215*(3): 403-410.

Anita, B. & Selvaraj, N. (2011). Biology, yield loss and integrated management of root-knot nematode, *Meloidogyne hapla* infecting carrot in Nilgiris. *Indian Journal of Nematology*, +2): 144-149.

Arun, A., Shanthi, A., Poornima, K., Arumugam, T., & Swarnapriya, R. (2019). Morphological and Molecular characterization of root knot nematode, *Meloidogyne incognita* associated with Cabbage and Cauliflower in Tamil Nadu. *Annals of Plant Protection Sciences*, *27*(3): 374-379.

Barker, K., & Campbell, C. (1981). Sampling nematode populations. *Plant parasitic nematodes*, *3*: 451-474.

Blok, V. C., Wishart, J., Fargette, M., Berthier, K., & Phillips, M. (2002). Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. *Nematology*, *4*(7): 773-781.

Bridge, J., & Page, S. (1982). The rice root-knot nematode, *Meloidogyne graminicola*, on deep water rice (*Oryza sativa* subsp. *indica*). *Revue de Nématologie*, *5*(2): 225-232.

Cenis, J. (1993). Identification of four major *Meloidogyne* ssp. by random amplified polymorphic DNA (RAPD-PCR). *Phytopathology-new york and baltimore then st paul*, *83*: 76-76.

Charchar, J. M., Eisenback, J. D., Vieira, J. V., Fonseca-Boiteux, M. E. d. N., & Boiteux, L. S. (2009). *Meloidogyne polycephannulata* n. sp.(Nematoda: Meloidogynidae), a root-knot nematode parasitizing carrot in Brazil. *Journal of Nematology*, *41*(3): 174.

Chitwood, B. (1949). 'Root-knot nematodes'. Part 1. A revision of the genus Meloidogyne Goeldi, 1887. In: Proceedings *of the helminthological Society of Washington*, *16*(2): 90-114.

Conceição, I. L., Tzortzakakis, E. A., Gomes, P., Abrantes, I. & Da Cunha, M. J. (2012). Detection of the root-knot nematode *Meloidogyne ethiopica* in Greece. *European Journal of Plant Pathology*, *134*(3): 451-457.

Cunha, T. G. d., Visôtto, L. E., Pinheiro, L. M., God, P. I. V. G., Rosa, J. M. O., Oliveira, C. M. G., & Lopes, E. A. (2021). Distribution of *Meloidogyne* species in carrot in Brazil. *Ciência Rural*, *51*.

Davis, R. M., & Nu, J. (2007). Integrated approaches for carrot pests and diseases management. *General Concepts in Integrated Pest and Disease Management*, Springer, pp. 149-188.

Douda, O., Manasova, M., Zouhar, M., Hnatek, J., & Stejskal, V. (2021). Field validation of the effect of soil fumigation of ethanedinitrile (EDN) on the mortality of *Meloidogyne hapla* and carrot yield parameters. *Agronomy*, *11*(2): 208.

Eisenback, J., Hirschmann, H., & Triantaphyllou, A. (1980). Morphological comparison of *Meloidogyne* female head structures, perineal patterns, and stylets. *Journal of Nematology*, *12*(4): 300.

Greco, N., Aranda, J. L., Saporiti, M., Maccarini, C., De Tommaso, N., & Myrta, A. (2018). Sustainability of European vegetable and strawberry production in relation to fumigation practices in the EU. IX International Symposium on Soil and Substrate Disinfestation 1270.

Hay, F. S., & Pethybridge, S. J. (2005). Nematodes associated with carrot production in Tasmania, Australia, and the effect of *Pratylenchus crenatus* on yield and quality of Kuroda-type carrot. *Plant disease*, *89*(11): 1175-1180.

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J., & Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular biology and evolution*, *23*(9): 1792-1800.

Huang, X., & Madan, A. (1999). CAP3: A DNA sequence assembly program. *Genome research*. 9(9): 868-877.

Joyce, S. A., Burnell, A. M., & Powers, T. O. (1994). Characterization of *Heterorhabditis* isolates by PCR amplification of segments of mtDNA and rDNA genes. *Journal of Nematology*, *26*(3): 260.

Kayani, M. Z., Mukhtar, T., Hussain, M. A. & Ul-Haque, M. I. (2013). Infestation assessment of root-knot nematodes (*Meloidogyne* spp.) associated with cucumber in the Pothowar region of Pakistan. *Crop Protection*, *47*: 49-54.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, *35*(6): 1547.

Medina-Canales, M. G., Terroba-Escalante, P., Manzanilla-López, R. H., & Tovar-Soto, A. (2019). Assessment of three strategies for the management of *Meloidogyne arenaria* on carrot in Mexico using *Pochonia chlamydosporia* var. mexicana under greenhouse conditions. *Biocontrol Science and Technology*, *29*(7): 671-685.

Mitkowski, N., Van der Beek, J., & Abawi, G. (2002). Characterization of root-knot nematode populations associated with vegetables in New York State. *Plant disease*, *86*(8): 840-847.

Nisha, M. S., Narayana, R., & Sheela, M. S. (2012). Occurrence of root-knot nematode, *Meloidogyne incognita* on carrot in Kerala. *Indian Journal of Nematology*, *42(*2): 194-196.

Pereira, J. D. S., Diamantino, M. S. A. S., de Oliveira, E. J., de Oliveira, S. A. S., & Abreu, E. F. M. (2016). Diagnose molecular de fitoplasmas e vírus associados ao couro-desapo da mandioca (Manihot esculenta Crantz). In Embrapa Mandioca e Fruticultura-Resumo em anais de congresso (ALICE). In: Jornada científica embrapa mandioca e fruticultura, 10., 2016: Cruz das Almas, BA. Traduzindo ciência para o mundo: resumos. Brasília, DF: Embrapa, 2016.

Potter, A. S., Foroudi, S., Stamatikos, A., Patil, B. S., & Deyhim, F. (2011). Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. *Nutrition Journal*, *10*(1): 1-6.

Ralmi, N. H. A. A., Khandaker, M. M.. & Mat, N. (2016). Occurrence and control of root knot nematode in crops: a review. *Australian Journal of Crop Science*, *11*(12): 1649.

Seenivasan, N. (2017). Status of root-knot nematode, *Meloidogyne hapla* infection on carrot at Kodaikanal hills of Tamil Nadu, India and its yield loss estimation. *Int. J. Curr. Microbiol. App. Sci*., *6*: 1-7.

Seesao, Y., Gay, M., Merlin, S., Viscogliosi, E., Aliouat-Denis, C., & Audebert, C. (2017). A review of methods for nematode identification. *Journal of Microbiological Methods*, *138*: 37-49.

Seo, Y., Kim, Y. S., Park, Y., & Kim, Y. H. (2015). Comparisons of pathological responses in carrot to root-knot nematodes. *The plant pathology journal*, *31*(4): 441.

Skantar, A., Carta, L., & Handoo, Z. (2008). Molecular and morphological characterization of an unusual *Meloidogyne arenaria* population from traveler's tree, *Ravenala madagascariensis*. *Journal of Nematology*. *40*(3): 179.

Sowmya, R., Kalaiarasan, P., Poornima, K., Swarnakumari, N., & Kannan, M. (2018). Species diversity of root knot nematodes infesting vegetable crops in Tamil Nadu. *Pest Management in Horticultural Ecosystems*, *24*(1): 58-65.

Wesemael, W. M., & Moens, M. (2008). Quality damage on carrots (*Daucus carota* L.) caused by the root-knot nematode *Meloidogyne chitwoodi*. *Nematology*, *10*(2): 261-270.

Ye, W., Robbins, R. T., & Kirkpatrick, T. (2019). Molecular characterization of root-knot nematodes (*Meloidogyne* spp.) from Arkansas, USA. *Scientific reports*, *9*(1): 1-21.

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