



Isolation and morphological identification of root knot nematode from cucumber roots

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Abstract

The root-knot nematodes are one of the major pests affecting the yield of many economically important annual and perennial crops worldwide in tropical and subtropical climatic zones. The infected plants show symptoms like root galling, stunted growth, yellowing of leaves, and wilting. The root-knot nematodes are one of the most destructive and difficult diseases to control in agricultural sector. In the present study, infected roots of cucumber plant containing root knot nematodes were brought from the cultivated fields of Pune district. Nematodes were isolated and identified based on morphological characters. The genus was found to be *Meloidogyne* and species *incognita*.

Keywords: root knot nematode, *Meloidogyne incognita*, cucumber, root galls

Introduction

Plant parasitic nematodes are one of the most dangerous pests of cultivated crops and are responsible for an average annual loss of 12.3 % in the world's major crops [4, 11]. They are obligate plant parasites that feed on roots and are able to parasitize almost every species of vascular plants [7]. The plant parasitic nematodes associated with vegetative crops are *Meloidogyne* spp., *Rotylenchulus reniformis*, *Heterodera* spp., *Pratylenchus* spp., *Helicotylenchus* spp. and *Hoplolaimus* spp. in India [1]. Root knot nematode invasion in roots results in altered root architecture. The most common morphological change is the characteristic root galling, which affects the uptake of water and nutrients. As a result of the damage to the root system, typical above ground symptoms of nematode infections include stunting, yellowing, and wilting, and yield reductions. Losses in the yield of crop due to root knot nematode may range between 10% to 80% depending on the nematode species, population level, and crop species [2]. The most important species of root-knot nematode *Meloidogyne* are *M. javanica*, *M. arenaria*, *M. incognita* and *M. hapla* that cause high economic damages in different crops [9]. Cucumber (*Cucumis sativus*) is an important vegetable crop, widely cultivated in India. Most cucurbits are extremely susceptible to root nematodes. Root-knot nematodes are one of the limiting factors in cultivated crops like fig, pomegranate, carrot, pigeon pea, cucumber and cotton in Maharashtra causing considerable yield losses [12]. Since there is lot of damage to the crop of cucumber in the area, the study was undertaken.

Materials and Methods

Source of root-knot nematodes

The root-knot nematode infected cucumber roots which showed galls were collected from a field in Pune district in the polythene bags.

Isolation of root knot nematode egg mass, juveniles and females from cucumber roots

The Root-knot nematode egg masses were collected from the cucumber roots by using forceps and also by using

NaOCl method combined with blending the roots in a blender at 30 sec interval for 3 minutes (Hussey and Barker, 1973) [5]. Egg masses were subsequently collected in a petri dish containing distilled water. They were incubated at 28°C for 48 hours to hatch second-stage juveniles (J2 stage). Mature females of root knot nematode were teased out from roots by using stereo zoom binocular microscope.

Identification of species

Identification of the species of root knot nematode is performed by perineal pattern method (Eisenback *et al.*, 1991) [3] and by morphometric analysis of different life forms i.e eggs, juveniles and females. Mature females were dissected out from the galls present in the roots. The perineal pattern were prepared by cutting the posterior end of the female, mounted on glass slide in a drop of anhydrous glycerine, covered with a coverslip and observed under the microscope.

Measurement of Nematode

The measurements were recorded using ocular micrometer at 40 X. The following measurements were recorded in different stages of root knot nematode i.e total body length (μm), maximum body width (μm) and stylet length (μm). The obtained data was compared with the earlier descriptions of this species (Kofoid and White (1919) [10], Jepson (1987) [6], Pal and *et al* (2013) [13] and Kshetrimayum *et al* (2018) [11]).

Results and Discussion

Morphology of Root

The roots showed gall formation (Fig1.A). The galls were small as well as massive. Scanty secondary roots were observed arising from the galls. (Fig1.B)

Morphology and Morphometric description of the Root Knot Nematode

Morphology:-Mature Female (n= 10) Fig.1.D

The body is globular to pear shaped and milky white, with a projecting neck and translucent cuticle. The neck has blurry annulations; the posterior part of the body is round and has

no protuberance. The stylet is slender and hard with round basal knobs. Excretory pore is located behind the level of stylet knobs.

Perineal pattern (Fig.1.E) is oval to round, high with squarish dorsal arch and distinct lateral lines are absent. The striae is wavy to zigzag. The vulval slit was straight. The length of perineal pattern was 113.22 μm and width 109.89 μm .

J2 Stage (n=10) Fig.1.F & G

Body is slender, the labial disc and lips are joined to form smooth and continuous structure. The head region shows annulations. Stylet slender with small rounded basal knobs. Tail is simple and tapers steadily to the terminus.

The perineal pattern analysis of the female nematode was typical of *Meloidogyne incognita* and the results of morphometric data of the mature females, J2 stage and eggs of *Meloidogyne incognita* were based on 10 females, 10 juveniles (J2) and 10 Eggs as shown in Table 1. They are compared with Kofoid and White (1919)^[10], Jepson (1987)

^[6], Pal and *et al* (2013)^[13] and Kshetrimayum *et al* (2018)^[11] in Table 2.

Meloidogyne incognita is one of the most ubiquitous species in the genus and has large host range. Galls produced by *M. incognita* are not the distinguishing factor for the species. Depending upon the population, development of galls take place, either it occurs singly or if the population is high, many galls merge to form large and sometimes massive knots. Less number of secondary roots emerge from the galls which is observed in our results as shown in Figure 1 (B), Similar observation has been made by Eisenback *et al.*, (1991)^[3]. According to Kaur and Attri (2013)^[8] perineal pattern is the most stable character of root-knot nematodes. Jepson (1987)^[6] has assessed that stylet length in juvenile was considered as differential character for identification of *Meloidogyne* species. Comparing the morphometric measurements obtained with Kofoid and White (1919)^[10], Jepson (1987)^[6], Pal *et.al* Maharashtra (2013)^[13], Kshetrimayum *et.al* Assam (2018)^[11], we confirm that the species of *Meloidogyne* is *incognita*.

Table 1: Morphometric characters of second-stage Juveniles (J2), Mature Females and Eggs observed in *M. incognita* found in cucumber roots [Mean \pm SD ; (range)], n = 10.

	Length (μm)	Body width (μm)	Stylet length (μm)
Mature Female	608.5 $\mu\text{m} \pm 47.02$ (530-680)	485.30 $\mu\text{m} \pm 47.83$ (390-560)	15.25 $\mu\text{m} \pm 0.74$ (14 -16.03)
Juveniles (J2)	390.68 $\mu\text{m} \pm 24.01$ (348-426)	13.54 $\mu\text{m} \pm 1.54$ (11.12 -15.18)	11.65 $\mu\text{m} \pm 0.93$ (10.13-13.00)
Eggs	87.99 $\mu\text{m} \pm 1.67$ (83.50-89.05)	38.66 $\mu\text{m} \pm 1.25$ (36-40)	-

Table 2: Comparison of the range of the morphometric data recorded from the mature females, juveniles and eggs of *M. incognita* from cucumber plant with Kofoid and White (1919), Jepson (1987), Pal and *et.al* (2013) and Kshetrimayum *et al* (2018).

Characters	Kofoid and White (1919)	Jepson (1987)	Pal <i>et.al</i> Maharashtra (2013)	Kshetrimayum <i>et. al</i> Assam (2018)	Present study
Females			n = 20	n = 15	n=10
Length	-	510-690 μm	560-690 μm	-	530-680 μm
Width	-	300-430 μm	420-590 μm	-	390-560 μm
Stylet Length	-	15-16 μm	14.25-16.15 μm	-	14.00-16.03 μm
JUVENILES					
Length	360-393 μm	337-403 μm	350-415 μm	360-478 μm	348-426 μm
Width	-	-	12.35-16.15 μm	-	11.12-15.18 μm
Stylet Length	10 μm	9.6-11.7 μm	11.40-12.35 μm	10.0-12.0 μm	10.13-13.0 μm
EGGS					
Length	-	-	85-88 μm	-	83.50- 89.05 μm
Width	-	-	35-40	-	36-40 μm

Conclusion

Perineal pattern of females is the most important morphological character for distinguishing the *Meloidogyne* species. Perineal pattern is oval to round, high with squarish

dorsal arch and distinct lateral lines are absent. Female perineal patterns of the root-knot nematode population in this study was typical of *Meloidogyne incognita*.

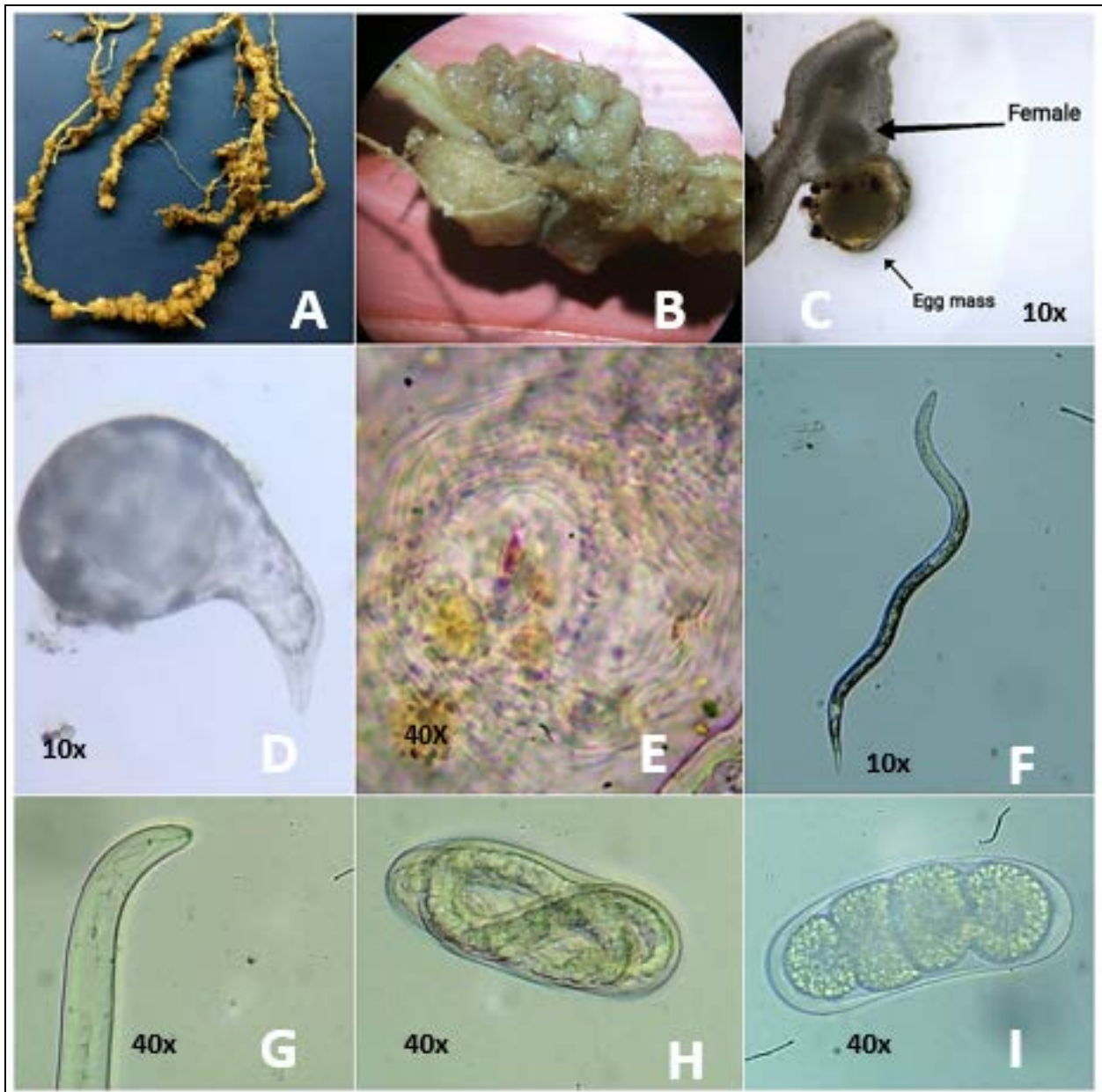


Fig 1: (A) Roots of Cucumber with galls (B) Cucumber root under stereozoom microscope showing scanty secondary roots arising from galls, (C) Cucumber root with female nematode and egg mass(10 x), (D) Mature Female observed under 10 x, (E) Perineal pattern 40x, (F-G) J2 stage of root knot nematode observed in 10x and 40x, (H)& (I) Eggs

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