

Studies on the Growth and Development of Root-Knot Nematode *Meloidogyne incognita* of *Phaseolous vulgaris* in Tissue Culture Medium

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Abstract: The life cycle of *Meloidogyne incognita* was observed and examined microscopically in the roots of *Phaseolous vulgaris* cv. Paulista. The nematode culture was maintained on MS media at $28\pm 2^\circ\text{C}$ in growth cabinet. The maximum number of *Meloidogyne incognita* J₂ that penetrated the root tissues of *Phaseolous vulgaris* was observed after 24 h from nematode infection. And the third stage juveniles were observed in root tissues after about 6-8 days from nematode inoculation. The pre adult females were seen inside root tissues within 20 days after nematode inoculation, whereas the adult females were seen inside roots within 27 days from nematode invasion. Egg laying females with deposited egg masses of *Meloidogyne incognita* were observed 37 days after nematode inoculation.

Keywords: *In vitro* culture, *Phaseolous vulgaris*, nematode, *Meloidogyne incognita*, root-knot nematode.

INTRODUCTION

The life cycle of root-knot nematodes *Meloidogyne* spp. is known to be affected by host plant, temperature, moisture and nutrition (Tarjan 1952 Bird and Wallace 1965, Bird 1970 and Taylor and Sasser 1987). Ibrahim *et al.*, (1973) observed the second stage juveniles of *M. incognita* inside rice roots within 24 hr, after nematode inoculation. However Thomson and Lear (1961) found that only few egg masses were produced by *Meloidogyne* spp. in 35 days at $15-16^\circ\text{C}$, and the number of egg masses produced increased rapidly at $20-21^\circ\text{C}$. However, the maximum egg masses production occurred for most species in the temperature range of $25-32^\circ\text{C}$. Veredjo *et al.*, (1988) studied the reproduction of *M. javanica* on *Agrobacterium rhizogenes*-transformed root cultures of several plants (potato, tomato, lima bean and carrot) under monoxenic condition. They found that the nematode was developed on all transformed roots at moderate rates. The *M. javanica* reared on transformed root cultures was able to complete its life cycle on new transformed root culture.

MATERIALS AND METHODS

- Preparation of the medium:

MS (Murashige and Skoog) medium was used to germinate the seeds of *Phaseolous vulgaris* cv. Paulista. The pH of the medium was adjusted to 5.8 by using 1.0 N HCl and 1.0 N NaOH, and agar was added after adjusting the pH. All used equipment and media were steam-sterilized for 20 min at 121°C

- Culture Preparation and Inoculation:

Seeds of *P. vulgaris* cv. Paulista were surface sterilized by immersion them in 70% ethanol for 3 min, followed by 5% Clorox (sodium hypochlorite) for 10 min and then washed twice with sterile water. Each seed was germinated under aseptic condition in a jar containing 20ml of the culture medium. Cultures were incubated at $28\pm 2^\circ\text{C}$ under 16 hrs illuminations (2000 lux, day light fluorescent tubes) in growth cabinet.

Twelve days after incubation the freshly hatching J₂ of root-knot nematode *Meloidogyne incognita* were collected and sterilized by 2.5% Clorox for 3 min and then washed with sterile distilled water. Nematode inoculum's were transferred to seedlings in jars at the rate of (500 J₂ /seedling). After the following intervals: 1, 2, 4, 6, 8, 10, 15, 18, 20, 25, 28, 30, 35 and 37 days from nematode incubation, root samples were collected and cut into small pieces, then nematodes killed and fixed in F.A.A solution. Roots were stained by acid fuchsin, washed in tap water. The stained roots were mounted in glycerin on glass slide and examined under the compound microscope.

RESULTS

The developmental plant stages on MS media was determined in Fig (1). The initial inoculum level of a pathogen affects the rate and (or) degree of infection in a host plant. This is important to consider when developing modified plants with tolerance or resistance to *M. incognita* under *in vitro* conditions.

The life cycle of *M. incognita* duration was about 37 days in root tissues of *P. vulgaris* cv. Paulista. The microscopical examination Fig (1) of infected root tissues revealed that the maximum number of *M. incognita* J₂ penetrated the root tissues of *P. vulgaris* after 24 h from nematode infection. And the third stage juveniles were observed in root tissues after about 6-8 days from nematode inoculation. While the pre adult females were seen inside root tissues within 20 days after nematode inoculation, the adult females were seen inside roots within 27 days from nematode inoculation. Egg laying females with deposited egg masses of *M. incognita* were observed 37 days after nematode inoculation.

DISCUSSION

The life cycle of root knot nematodes *M. incognita* was studied by many investigators, and they found that the life cycle is usually affected by the host plant, temperature, moisture and nutrition. The results

obtained in the present study indicated that *M. incognita*, reared on *P. vulgaris* cv. Paulista seedlings on MS medium was able to complete its life cycle in the seedlings roots on tissue culture medium. The second stage Juveniles of *M. incognita* was able to penetrate the plant root within 24 hr this result is agreement with Ibrahim *et al.*, 1973. In addition, *M. incognita* was able to complete its life cycle within 37 days on MS medium

at 28°C. This result is in disagreement with the results obtained by Thomson and Lear, 1961. Our success in studying the life cycle of *M. incognita*, using tissue culture techniques encourages the study of other life cycles of plant parasitic nematodes. Especially when taking into consideration the difficulties of such studies in soil.

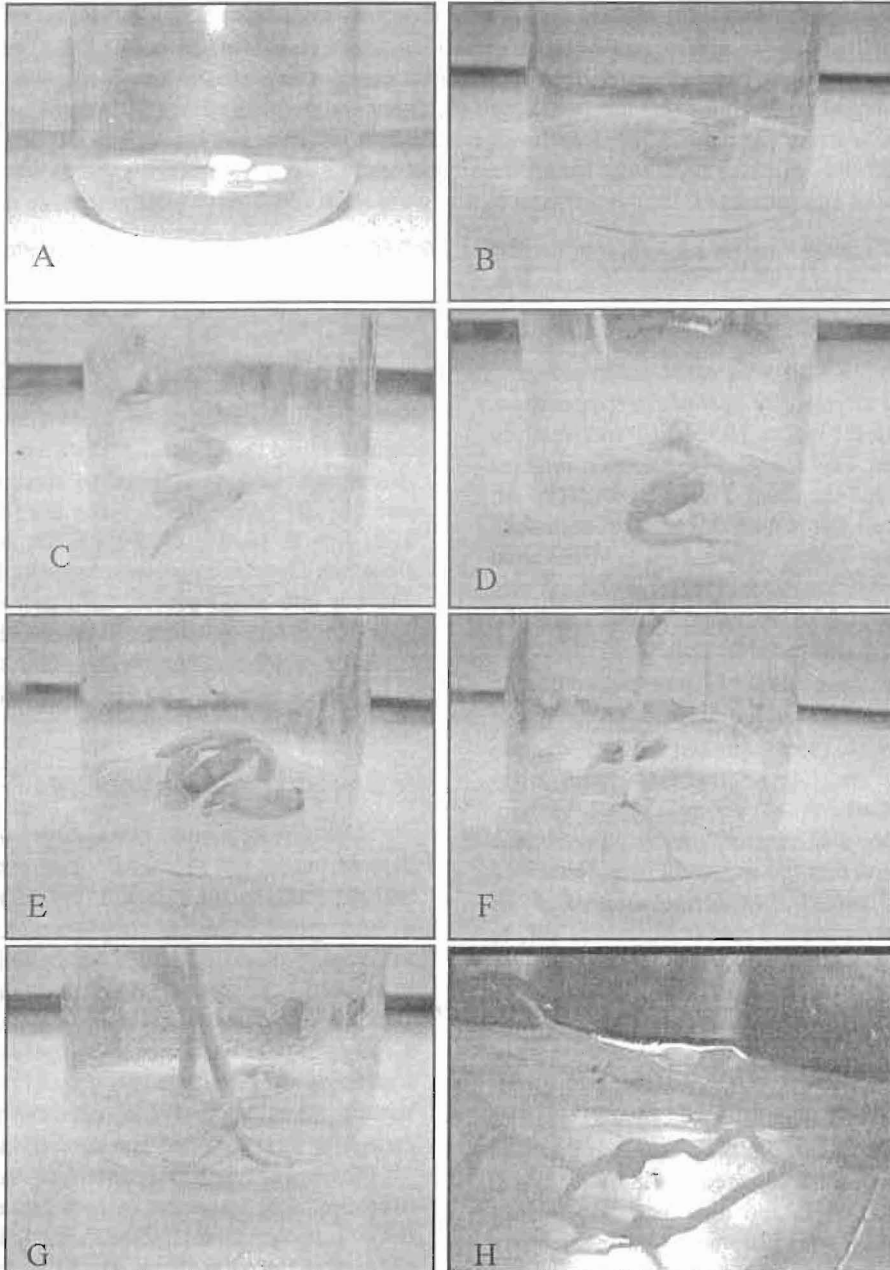


Fig. (1): The developmental plant stages on MS media.

- A: Seeds on MS media
- B: Seed swelling
- C: Radical appearance
- D-E: Cotyledon leaves appearance
- F-G: Seedlings
- H: Galls on the root

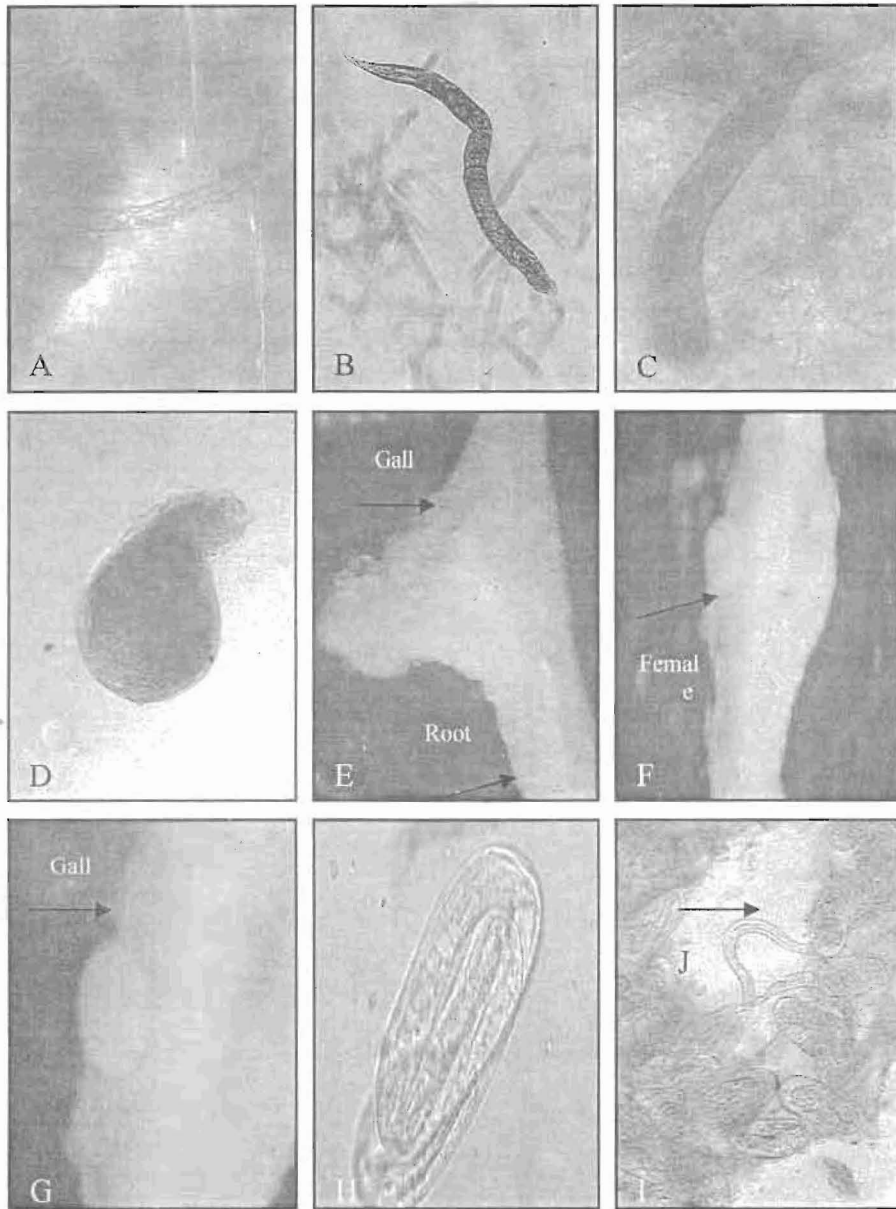


Fig. (2): The nematode developmental stages on bean roots on MS media

A: J₂ penetrate the root B: J₂ in the root C: J₃ in the root
 D: pre adult female E: egg mass F: female
 G: female inside root H: J₂ inside the egg I: egg hatching

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دراسة نمو وتطور نيماتودا تعقد الجذور *Meloidogyne incognita* على نباتات الفاصوليا في بيئة زراعة الانسجة

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أظهر الفحص الميكروسكوبي لدورة حياة نيماتودا تعقد الجذور جنس *Meloidogyne incognita* والمنماه على جذور نباتات الفاصوليا صنف بوليسستا في بيئة زراعة الانسجة وعلى درجة حرارة ٢٨ °م بغرفة الزراعة ان غالبية اعداد الطور اليرقي الثاني (الطور المعدى) اخترقت جذور النباتات بعد ٢٤ ساعة من العدوى. وظهر الطور اليرقي الثالث بداخل الجذور بعد ٦-٨ ايام من العدوى. في حين شوهدت الاناث غير الناضجة بداخل الجذور بعد ٢٠ يوم من العدوى بالنيماتودا. والاناث الواضحة للبيض لوحظت بداخل الجذور بعد ٣٧ يوم من العدوى بالنيماتودا. وأهمية هذه الدراسة أنها تشجع دراسة النيماتودا المعروفة بتطفلها الإجبارى معمليا.