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Open Access Journal of Mycology & Mycological Sciences
ISSN: 2689-7822

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

Abdel Monaim MF[1]*, Abdel Baset SH[2], Massoud SI[3], Abdelmoem TS[3] and El Hamawi MH[2]

[1]Leguminous Crops and Fodder Diseases Research, Plant Pathology Research Institute, Egypt

[2]Department of Nematode Diseases Research, Plant Pathology Research Institute, Egypt

[3]Department of Agricultural Botany, Suez Canal University, Egypt

Research Article

Volume 6 Issue 1

Received Date: March 10, 2023

Published Date: April 17, 2023

DOI: 10.23880/oajmms-16000174

***Corresponding author:** Montaser F Abdel-Monaim, Department of Legume Diseases Research, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt, Email: fowzy_2008@yahoo.com

Abstract

The study was conducted to study the growth and development of root-knot nematode, *Meloidogyne incognita* of *Phaseolus vulgaris* in tissue culture media. The data revealed the developmental common bean cv. Paulista of M.S media as the following: the seed swelling stage after three days, the radical appearance stage after five days. The cotyledons leaves appear after six days, the cotyledon leaves appear after eight days, and the seedling stage after twelve days. The life cycle of *M. incognita* completed about thirty-seven days in root tissues of Paulista cultivar. Microscopic examinations of the infected bean roots revealed the following: Second-stage juveniles of *M. incognita* penetrated the roots within 24 hrs after nematode inoculation. Third-stage juveniles were observed in roots (six-eight) days after nematode inoculation. While, fourth stage juveniles were observed in roots 12-14 days after nematode inoculation. The pre-adult females were seen inside roots within twenty days after nematode inoculation. The adult females were seen inside roots within twenty-seven days after nematode inoculation. Egg laying females with deposit egg masses of *M. incognita* was observed thirty-seven days after nematode inoculation.

Keywords: *Meloidogyne incognita*; Life Cycle; *Phaseolus vulgaris*; Tissue Culture

Introduction

Common bean (*Phaseolus vulgaris* L.) is an economically important crop and one of the major grain legumes for human consumption in Latin America, Africa and Asia [1,2]. Despite its importance, production growth rates are limited by viral, nematode, fungal, and bacterial pathogens, insects, lack of drought tolerances and nutritional deficiencies [3]. Therefore, there is considerable interest in the development of new bean cultivars with useful agronomical traits [3]. Plant biotechnology, together with conventional breeding methods, could facilitate common bean improvement since resistance or tolerance to biotic and abiotic stress could be increased and seed quality, plant architecture, and reproduction modes could be altered [4]. Nevertheless, a reliable and efficient in vitro culture Root-knot nematodes invade an array of important crops and have been found more damaging to vegetables, especially in the tropical and subtropical countries of the world [5]. So far, more than 100 species of *Meloidogyne* have been described throughout the world and, among these, four *species* viz. *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla*, which are

commonly found. More than 3000 plant species, almost all cultivated plants, have been recorded as the hosts of root-knot nematodes. The infested plants manifest symptoms of chlorosis, stunting, and unthrifty growth [6].

The life cycle of *Meloidogyne* includes six developmental stages: the egg stage, four juvenile stages (J1–J4), and the adult stage. The life cycle begins when the only infective and mobile stage, J2, penetrates the roots. Then, the nematode migrates through intercellular spaces to establish a permanent feeding site in the vascular cylinder, in which it will induce the differentiation of five to seven cells, named giant cells, that experience morphological, physiological, and molecular modifications caused by the J2 infection and will supply food to the nematode. The root tissue becomes distorted due to the hyperplasia around the cell, forming the root gall. Once the nematode establishes its feeding site, it will become sedentary and will grow in length and width (J3–J4), until reaching the adult stage. In favorable conditions, J2 develops in pear-shaped females. They lay eggs into a gelatinous matrix (egg mass), generally outside the gall. Embryogenesis will lead to a J1 developing inside the egg, which will molt to J2 that will emerge and migrate into the soil. Only males appear under unfavorable conditions, and they will migrate into the soil with no evidence of feeding on plants [7].

Plant tissue cultures have been utilized to propagate some species of tissue invading nematodes. In most studies on *Meloidogyne* spp., investigators have employed larvae or eggs from roots growing in infested soil. This technique is a simple, inexpensive method for maintaining an abundant supply of *M. incognita* in the laboratory. By utilizing glass capillary tubes for inoculations, single egg sacs can be isolated to establish and perpetuate pure nematode cultures. Most of the protocols for *in vitro* regeneration of common bean are based on direct organogenesis or shoot development of different types of exploits, *viz.* shoot apical meristems, cotyledonary and primary leaf node explants, petiole etc. [8,9]. However, these procedures yield very low regeneration efficiency [10]. Another approach has been done through indirect in vitro regeneration. To date, there are only four reports on indirect regeneration of *P. vulgaris*. However, the frequency of shoot regeneration from callus is extremely low [11,12] or higher depends on cultivars [13,14]. Therefore, the objective of the present work was to study the growth and development of root-knot nematode *Meloidogyne incognita* of *Phaseolous vuligaris* in tissue culture media.

Materials and Methods

In vitro experiments were conducted at the Department of Agricultural Botany, Faculty of Agric., Suez Canal Univ. The cultivar cv. Paulista was obtained from the Vegetable Crop Res. Depar., Horticul. Res. Inst., Agric. Res. Center, Giza. Culturing of Nematode: The pure culture of *M. incognita* from single egg mass has been maintained on tomato seedling (*Lycopersicon esculantum* Mill cv. G.S) at 25±2°C in order to obtain sufficient numbers of second stage juveniles.

Preparation of the Medium

Murashige [15] a medium protocol multiplication were used. The pH of medium was 5.8 by using 1.0NHCLand 1.0 NaOH, and agar was added after adjusting the pH. All used equipment and media were autoclaved for 20 min at 121°C.

Culture Preparation and Inoculation

Seeds of *P. vulagris* cv. Paulista were surface sterilized by immersing them for 3min in 70% ethanol, followed were surface sterilized with 0.1 sodium hypochlorite (NaOCl) [16] for 10 min and then washed twice with sterile water. Each seed was germinated under aseptic condition in a jar containing 30 ml of the culture medium. Culture was incubated at 28±2° C under 16 hrs photo periods in growth cabinet. Twelve days after incubation, the freshly hatching second stage juveniles of root-knot nematode (j2) were collected and sterilized by 2.5% Clorox for 3 min and then washed with sterile distilled water twice. Finely drawn capillary tubes (1 mm in diameter and 50 mm in length) were used to pick up the concentrated larval suspensions from the depression slide preparations. Using 100 X magnification, the larvae drawn into each capillary tube were counted. Glass capillary tubes containing about 500 j2s /seedling were used to inoculate the host plant (common bean). Capillary tubes containing larvae were inserted into the root. All inoculated plants were grown under greenhouse conditions. Observations were made for at least 2 weeks on all plants. Some plants were examined and studied for intervals: 1, 2, 4, 6, 8, 10, 15,18,20,25,28,30,35, and 37 days following nematode incubation. Microscopic examinations of fresh and fixed sections were made periodically. For the demonstration of nematodes in roots, sections were prepared and stained according to the methods of [17,18].

Results and Discussion

Developmental Stages of *in Vitro* Propagation of Common Bean Bean Cv. Paulista by Direct Organogenesis from the Embryonic Axis of Mature Seed

The developmental common bean cv. Paulista plant stages of M.S media revealed in Figure 1 as the following: the seed stage in Figure 1A, the seed swelling stage after 3 days Figure 1B, the radical appearance stage after 5 days Figure 1C. The cotyledons leaves appear after 6 days Figure 1D, the

cotyledon leaves appear after 8 days Figure 1E, the seedling stage after 12 days Figure 1F. The use of tissue culture for plant regeneration and the introduction of foreign genes that confer pathogens resistance or drought tolerance are good options for bean cultivation. Most of the published protocols are based on direct organogenesis or shoot development from meristematic cells [11].

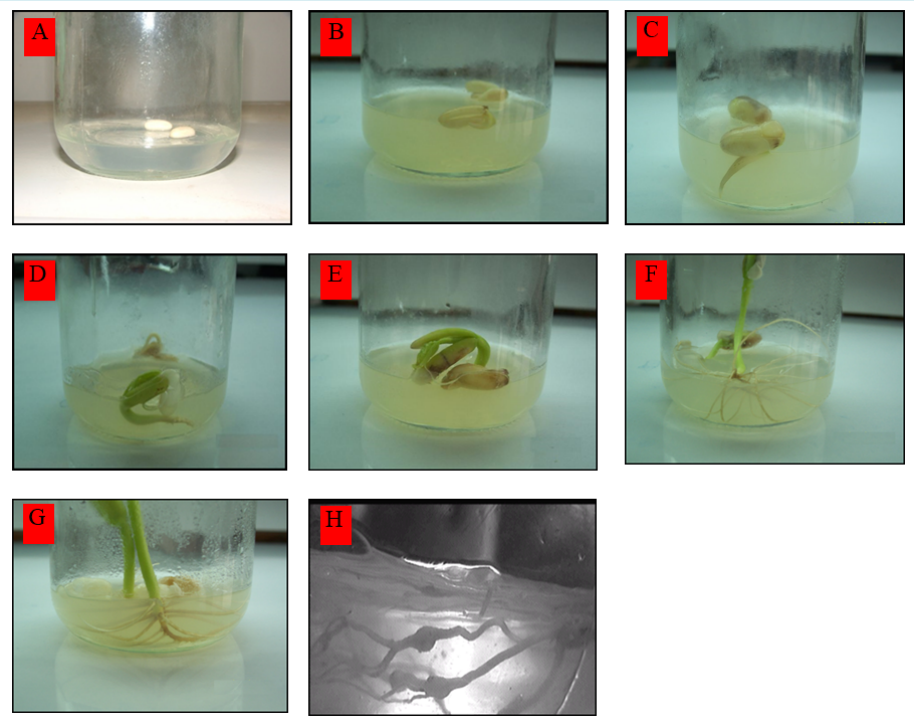

Figure 1: The developmental plant stages of common bean cv. Paulista on M.S. med.
A: Seeds on M.S media; B: Seed swelling; C: Radical appearance; D: Cotyledon leaf appearance; E: Cotyledon leaf appearance; F: Seedlings; H: Galls on the root

Some recent examples of direct organogenesis may be found in the literature [10]. Until now several types of cells, tissues and organs (cotyledonary nodes, embryonic axes, auxiliary shoots, cotyledon with split embryo axis, internodes, hypocotyls, leaves, leaf petioles or intact seedlings) have been used to induce the regeneration pathways [1,12,19]. Cotyledons with part of the embryonic axis as explained have been used to regenerate whole plants [20]. These protocols are very efficient.

Life cycle and Developmental Stages of *M. incognita* on Tissue Culture

The life cycle of *M. incognita* completed about 37 days in root tissues of *P. vuligaris* cv. Paulista. Microscopic examinations of the infected bean roots revealed in Figure 2 as the following: Second stage juveniles of *M. incognita* penetrated the roots within 24 hr after nematode inoculation Figure 2A. Second–stage juvenile (j2) observed in roots 2-3 days after nematode inoculation Figure 2B. Third stage juveniles were observed in roots 6-8 days after nematode inoculation. Figure 2C. Fourth stage juveniles were observed in roots 12-14 days after nematode inoculation Figure 2D. The pre adult females were seen inside roots within 20 days after nematode inoculation Figure 2E. The adult females were seen inside roots within 27 days after nematode inoculation. Figure 2F. Egg laying females with deposit egg masses of *M. incognita* were observed 37 days after nematode inoculation.

Many investigators reported that there were many factors that affected the life cycle of *Meloidogyne* spp., the results obtained in the present study indicated that *M. incognita*, which reared on transforming the root culture was able to complete its life cycle on new transformed root tissue culture.

Data illustrated that *M. incognita* completed its life cycle in 37 days on full M.S medium. Second-stage juveniles of *M. incognita* penetrated the roots within 24 hr from inoculation. The adult females were seen inside roots within 27 days after nematode inoculation, and completed the life cycle in 37 days on full M.S medium. These results are in agreement with

[21]. While [22] mentioned that Belonolaimus longicaudatus, completed three molts within 29 days on B5 medium. The benefit of using root tissue culture for studying the life cycle of *M. incognita*, in vitro as obligated indo parasite on bean plant, while encourage studying other life cycles of plant-parasitic nematodes either ecto or endo parasite in a laboratory and developing suitable medium for each taking into our consideration the difficulties of such studies in soil.

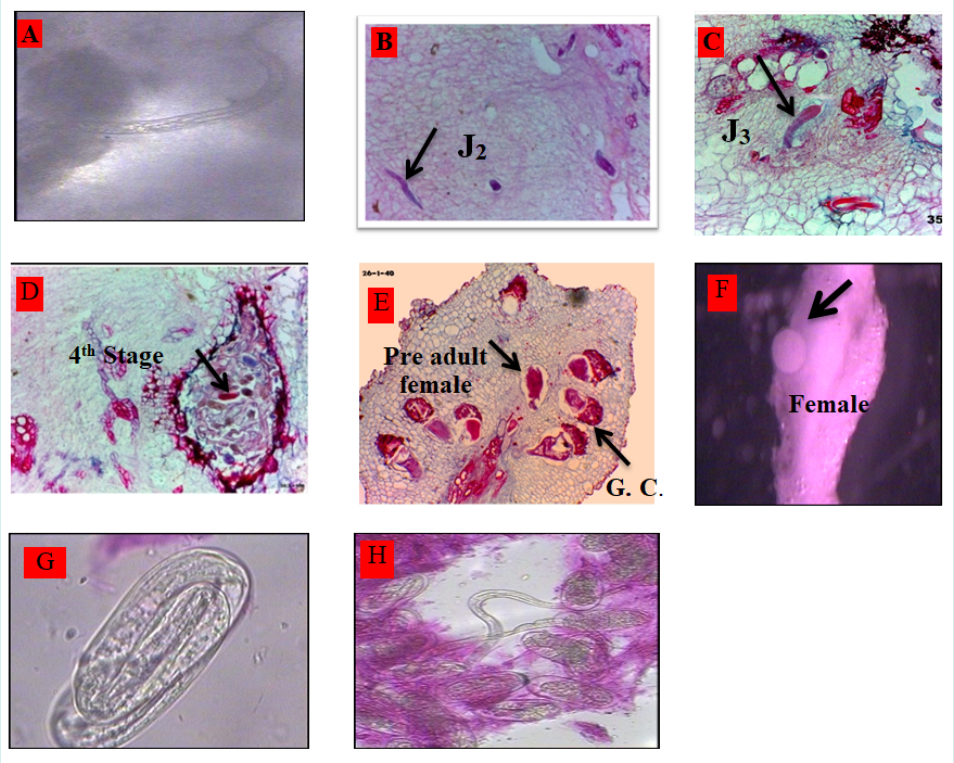

Figure 2: The nematode developmental stages *M. incognita* on bean roots on M.S. media (×100).
A: J2 Penetrate The Root; B: J2 In The Root; C: J3 In The Root; D: 4th Stage Juvenile; E: Pre Adult Female And Giant Cell (G.C); F: Female; G: The Egg; H: Egg Hatching.

Conclusion

Root-knot nematode, *Meloidogyne incognita* completed its life cycle about thirty seven days in root tissues of Paulista cultivar on tissue culture media. Second-stage juveniles of *M. incognita* penetrated the roots within 24 hrs., and third-stage juveniles were observed in roots (six-eight) days after nematode inoculation. Fourth-stage juveniles were observed in roots 12-14 days, and the pre-adult females were seen inside roots within twenty days after nematode inoculation. The adult females were seen inside roots within twenty-seven days after nematode inoculation. While, egg laying females with deposit egg masses of *M. incognita* was observed thirty-seven days after nematode inoculation.

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