

## Development of *Meloidogyne incognita* in Muskmelon (*Cucumis melo* L.)

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(Received: June 20, 2024; Accepted: November 28, 2024)

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### ABSTRACT

Root-Knot Nematodes (RKNs) are destructive plant parasites, known for causing characteristic galls in plant roots that reduce crop yields and crop quality. This study focuses on *Meloidogyne incognita*, a major RKN species affecting muskmelon crops. The primary objective is to examine the embryonic development of *M. incognita* eggs and the process of penetration and maturation within muskmelon (Arka Siri) plants under greenhouse conditions. The embryonic development of *M. incognita* eggs was monitored using the hanging drop technique, documenting their transformation from single-cell stage eggs to second-stage juveniles over time. Root penetration analysis revealed that second-stage juveniles (J<sub>2</sub>) penetrated near the root tip region just a day after inoculation. These invaders progressed through the root tissues, feeding on both cortical and vascular tissues. Subsequent molting events led to the emergence of third-stage (J<sub>3</sub>) and fourth-stage (J<sub>4</sub>) juveniles. As time passes, immature females matured into egg-laying adults, completing the life cycle within 28-30 days in muskmelon, where temperature (25-30 °C) plays an important role. This investigation provides valuable insights into *M. incognita*'s life cycle in Muskmelon plants, enhancing our understanding of the host-parasitic mechanism employed by RKNs. These findings help in developing effective strategies to combat RKN infestations, ultimately improving crop yields and overall crop quality.

**Key words:** *Meloidogyne incognita*, embryonic development, penetration, molting, life cycle, Muskmelon

### INTRODUCTION

Root-Knot Nematodes (RKNs) are the predominant plant parasitic nematodes that have a significant presence worldwide and their presence can be easily recognized by the characteristic galls formed through cell enlargement and rapid cell divisions (Sasser and Freckman, 1987). By interfering with anchorage and absorption of plant nutrients from soil in their host plants, root-knot nematodes reduce the host's yield and affect the marketable quality of economically important plant products (Abad et al., 2003). Furthermore, the altered metabolism induced by these nematodes weakens the host plant's defense against other pathogenic microorganisms, resulting in the formation of disease complexes and exacerbating the severity of the disease (Nelson, 2005).

RKNs, specifically belonging to the *Meloidogyne* genus, are polyphagous obligate sedentary endoparasite. They rely on feeding sites induced in the roots to complete their life cycle. The second stage juvenile (J<sub>2</sub>) penetrates the root system, migrates through intercellular spaces, and establishes a permanent feeding site in the stelar region (Escobar et al., 2015). The J<sub>2</sub> then undergoes

three successive molts to develop into third (J<sub>3</sub>) and fourth (J<sub>4</sub>) stage juveniles, eventually maturing into adults. The mature adult female lays eggs in a gelatinous matrix attached to the nematode's posterior end (Ibrahim et al., 1973). Infected roots often exhibit separation of vascular elements from the main bundles. The giant cells shift their metabolic focus towards protein synthesis and may hinder the transportation of nutrients to the rest of the plant (Bird, 1979). Galls formed due to root-knot nematode infestations also lead to the breaking and deformation of vascular elements, ultimately obstructing the translocation of water and nutrients. Consequently, the efficiency of the root system is compromised, resulting in diminished growth and yield because of the reduced and distorted roots (Dropkin et al., 1969).

Muskmelon (*Cucumis melo* L.) is a significant cucurbitaceous vegetable crop, widely cultivated for its delectable fruits. The fruits are rich in carbohydrates, vitamins A and C, and their seeds are also consumed after removing the seed coat. Due to their high nutritional value and consumer preference for healthy fruits, the demand for muskmelon has been increasing (Sunita and Hosakatte, 2013). However, RKNs pose a major obstacle to muskmelon production worldwide (Lamberti, 1979; Sasser,

1979; Di Vito et al., 1983; Netscher & Sikora, 1990). *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* are the most prevalent RKN species, all causing root galling and reducing muskmelon yields (Zitter et al., 1996). In this study, we aimed to investigate the embryonic development of *M. incognita* eggs and the penetration and development of juveniles in muskmelon.

## MATERIALS AND METHODS

The study on the biology of *M. incognita* was conducted as a pot culture experiment under controlled glasshouse conditions at the Department of Studies in Botany, Manasagangotri, University of Mysore, Mysuru. To propagate the root-knot nematodes, *M. incognita*, they were isolated from infected Muskmelon plants and transferred to two kg capacity earthen pots filled with sterilized pot culture using two-week-old tomato plants.

### Identification of *M. incognita*

Muskmelon roots infected with the root-knot nematode were collected from the field, washed, and stained with acid-fuchsin lactophenol solution (Byrd et al., 1983). After cooling to room temperature, the roots were rinsed with tap water and then soaked in plain lactophenol solution overnight for destaining. The roots were dissected under a stereo-zoom microscope, and adult female nematodes were extracted and preserved in lactophenol. The perineal sections of the nematodes were examined under a microscope, and 15 perineal patterns were compared with descriptions provided by Seinhorst (1966) and Eisenback (1985).

### Embryonic Development of *M. incognita*

The embryonic development of *M. incognita* eggs was studied using the hanging drop technique in the laboratory (Gilarte et al., 2015). Egg masses of *M. incognita* were detached from the roots of muskmelon plants 30 days after inoculation. A single egg was then transferred to a cavity block filled with distilled water and kept at room temperature (25-300 °C) and replicated thrice. The egg on the cavity slide was observed under a compound microscope, and observations were made at one-hour intervals for the first 2 days and thereafter at 6-hour intervals until the eggs hatched, with the developmental stages being documented (Gilarte et al., 2015).

### Root Penetration and Development of *M. incognita*

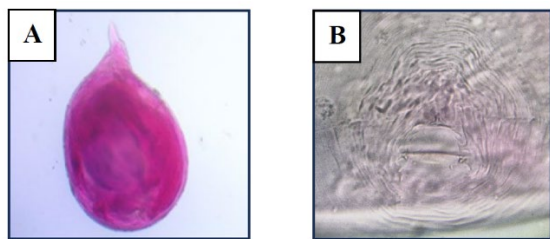
Root penetration and post-penetration developmental studies were conducted under glasshouse conditions where, temperature of the glasshouse was maintained between  $28 \pm 50$  °C. Roots with egg masses were removed from the soil two months after infestation on tomato plants, and the eggs were extracted using the sodium hypochlorite method as described by Hussey and Barker (1973). The egg masses picked were hatched in a beaker filled with sterile distilled water at  $28 \pm 20$  °C in the incubator. The newly emerged J<sub>2</sub> (second-stage juveniles) were collected after 24 hours and concentrated. Around 2000 newly emerged J<sub>2</sub> were placed around the root zone of a week-old seedlings of muskmelon (Arka Siri), with a ratio of 1 J<sub>2</sub> per gram of soil, and then watered. Three replications were maintained for each time interval. The plants were carefully uprooted at intervals of 1, 2, 4, 6, 8, 10, 12, 14, 21, and 30 days after inoculation, and the roots were thoroughly washed to remove soil, and stained with acid-fuchsin (Byrd et al., 1983; López-Gómez et al., 2014).

The post-embryonic stages of *M. incognita* (J<sub>2</sub>, J<sub>3</sub>, and J<sub>4</sub> stages, as well as adults) were studied by dissecting them from the plant tissue and mounting them in glycerine on glass slides for observation under a microscope. The occurrence of each molt was used to describe the time interval for each developmental stage (Eisenback et al., 1985). This process was continued until the nematode's life cycle was completed.

## RESULTS

### Identification of *Meloidogyne incognita*

Adult females of root-knot nematode were randomly selected from muskmelon roots for identification, employing the posterior cuticular pattern as the basis (Eisenback, 1985). The perineal pattern of all the root-knot nematode females exhibited typical characteristics, including a high squarish dorsal arch with a distinct whorl in the tail terminus, smooth wavy striae, striae forks, and a break in the lateral lines. Consequently, the nematode was identified as *M. incognita* using the conventional method of analyzing the posterior cuticular pattern (Figure 1B).



(A) Adult Female Nematode

(B) Perineal pattern

Fig. 1. Morphological characteristics *Meloidogyne incognita* for Identification.

### Embryonic Development of *Meloidogyne incognita*

The embryonic development of *M. incognita* eggs was investigated using the hanging drop technique in a controlled laboratory condition.

Under a compound microscope, the observed egg showed either an undifferentiated mass of cells or a single-celled stage (Figure 2A). The single-stage cell began dividing, and the first cleavage occurred within 3 to 4 hours. This cleavage took place transversely to the longitudinal axis, resulting in two equal cells or blastomeres, one being the first somatic cell and the other the parental germinal cell (Figure 2B). The second cleavage occurred approximately 8-10 hours after the single-stage cell, leading to the formation of four cells. Initially, these four cells were arranged in a T-shape, but later they developed into a rhomboid shape (Figure 2C, D). Subsequently, within 20-22 hours after the single-cell stage, further division occurred, resulting in the eight-cell stage (Figure 2E).

The cells continued to divide, and the multicell stage was achieved within 30-32 hours after the single-cell stage (Figure 2F). Following the multicell stage, subsequent developmental stages such as the blastular and gastrular stages were observed 9-10 days after the single-cell stage. At this point, two folded and three folded stages were visible within 11-12 days from the initial single-cell stage (Figure 2G,H). As the nematode continued to develop within the egg, it developed to form the first-stage juvenile ( $J_1$ ) on the twelfth day within the egg membrane (Figure 2I). As time progressed, the first-stage juvenile molted to form a second-stage juvenile ( $J_2$ ) which then hatched out from the eggshell by making repeated thrusts at the eggshell within 13-15 days after the single-cell stage egg under laboratory conditions of around 25-30 °C (Figure 2J).

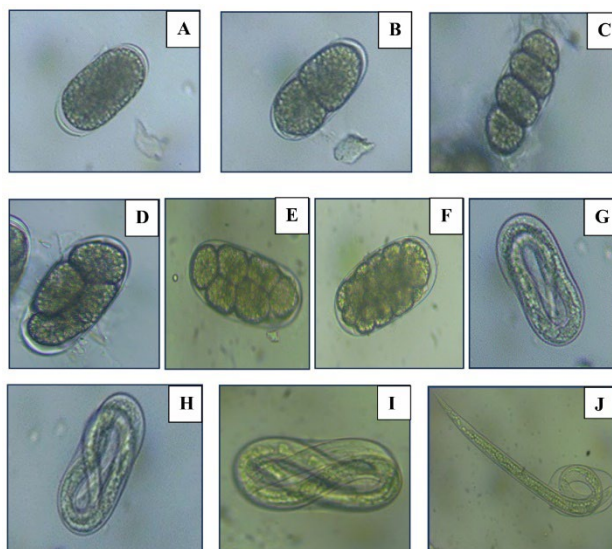


Fig. 2. Embryonic Development stages of eggs of Root-Knot Nematode *Meloidogyne incognita*. (A) Single-celled stage egg. (B) Two-celled stage egg. (C,D) Four-celled stage egg. (E) Eight-celled stage egg. (F) Multicell stage Egg. (G) Two-folded egg stage. (H) Three folded egg stage. (I)  $J_1$  within egg. (J)  $J_2$  excluding from eggshell.

### Root Penetration and Development of *Meloidogyne incognita*

Inoculated  $J_2$ 's were seen in the root-tip region merely a day after inoculation into muskmelon roots. However, after 48 hours  $J_2$ 's were seen behind the root tip, with some already inside (Figure 3B). These juveniles resembled those that emerged from the eggs used for inoculation. Starting from the third day after inoculation, most of the juveniles were found in the stele, although some were still entering the roots (Figure 3C). Their growth in size became prominent by the fourth day. Second molting was initially noticed on the fifth day, marking the transition to the third-stage juvenile ( $J_3$ ) (Figure 3D). A third molt took place on the seventh day after inoculation, leading to the emergence of fourth-stage juveniles ( $J_4$ ). This stage revealed two distinct juvenile types; one destined to become females (Figure 3E), and the other would develop into adult male. Females were abundant in the root, while only one of the male nematode was observed. The fourth molting event occurred on the ninth day after inoculation, giving rise to a young female nematode. By the twelfth day after inoculation, at terminal portion of the female egg mass is visible (Figure 3G). The nematode's head was attached to the root's stele, and other body parts were observed in the cortex. By the fifteenth day after inoculation, the developing females had begun producing a gelatinous matrix or egg sac, initially without eggs. Eggs were first observed in the egg sac on the

eighteenth day after inoculation, which were deposited into the egg mass as the single-celled stage or two-celled stage eggs (Figure 3G). A mature adult female nematode laid an average of  $367 \pm 10.5$  eggs, with these eggs measuring an average length of  $91.85 \pm 3.5 \mu$  and a width of  $38.45 \pm 2.4 \mu$  ( $n = 10$ ) and lacking uniformity in the overall shape.

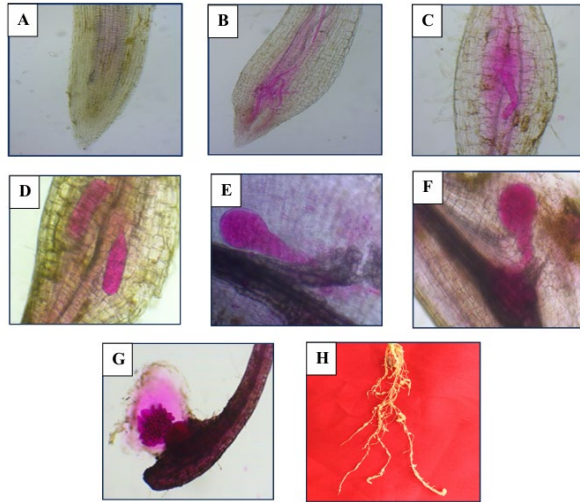


Fig. 3. Post-Embryonic development stages of *M. incognita* inside the roots of muskmelon. (A) Root tip without nematode penetration. (B) Penetration of J<sub>2</sub> in the root near root tip. (C) Migration of J<sub>2</sub> through cortex. (D) Spike tail stage of larvae—J<sub>3</sub>. (E) Immature female within roots. (F) Mature female within the root. (G) Egg-laying female with egg mass. (H) Muskmelon Roots with knots/galls.

Monitoring the development of males proved challenging, as only one adult male was observed during the entire experiment, aside from a single fourth-stage juvenile (J<sub>4</sub>) seen on the fourteen days after inoculation. The appearance of the male nematode inside muskmelon roots was observed 20-22 days after inoculation (Figure 3F). This male had a well-developed stylet with knobs, and pair of spicules were positioned at the rear end. The life stages of *M. incognita* on muskmelon (Arka Siri) are given in Tables 1 and 2.

**Table 1.** Embryonic developmental stages of *M. incognita* eggs.

Single-cell stage	1-2 hours
2 cell stage	3-4 hours
4 cell stage	8-10 hours
8 cell stage	20-22 hours
Multicell stage	30-32 hours
Blastula	9 days
Gastrula	10 days
2-fold stage	11 days
3-fold stage	12 days
J <sub>2</sub> stage	13-15 days

**Table 2.** Post-embryonic development of *M. incognita* in Muskmelon.

Stages	Duration
<b>Embryonic development</b>	
Single-cell to 2nd stage Juveniles	13-15 days
<b>Post-infection stage</b>	
2nd stage juvenile	16-17 days
3rd stage juvenile	18-19 days
4th stage juvenile	20-22 days
Swollen female	23-24 days
Fully developed female	25-27 days
Egg laying	28-30 days

## DISCUSSION

The morphological characteristics documented in this study (egg, juvenile, male, and female) are very similar to *M. incognita* characterized by Taylor and Sasser, (1978). Observation of the life cycle of *M. incognita* revealed that could complete its life cycle i.e., from single-celled egg stage to formation of egg mass from adult female within 28-30 days in muskmelon (Arka Siri), where temperature plays an important role in completing the life

cycle. Any change in optimum (25-30 °C) temperature can increase or decrease the duration of the life cycle, similar findings were reported by Joshi et al. (2019) and Abad et al. (2008).

The eggs of *M. incognita* are ellipsoid, and lack uniformity in the overall shape of the egg. The same kind of observations are seen in several root-knot nematodes (Lahl et al., 2004; Schierenberg, 2005) and it suggested that intra-specific differences might be as great as inter-specific variations described by Bird and Bird,

(1991). Single-celled stage eggs of *M. incognita* initially have a soft chorion before they are deposited into the external environment, after that only the chorionic shells harden. The vulva of the female compresses the egg when it is deposited into the gel matrix and this physical action may trigger to initiate the development.

In this study, we observed single-celled stage eggs of *M. incognita* transferred through the reproductive tract of the female, which was deposited into the egg mass as single-celled stage or two-celled stage eggs. Similar observations are made by Calderón-Urrea et al. (2016) in *M. incognita*, and also in *M. enterolobii* by Ashokkumar et al. (2019). As the nematode develops in the egg, which begins within a few hours after deposition into egg mass, resulting 2,4,8 and so on until a fully formed first-stage juvenile with a visible stylet lies coiled inside the egg membrane which molts to form second-stage juvenile (J<sub>2</sub>) which hatches out from the eggshell, where J<sub>2</sub> is the only infective stage juvenile that penetrates and forms the galls in the roots. These findings are similar to the results given by Kavitha et al. (2011) in Noni plants, the same findings were also given by Taylor and Sasser, (1978). The second-stage juveniles (J<sub>2</sub>) move toward vulnerable roots by detecting chemical gradients released from the host root through their chemosensory amphids (Teillet et al., 2013).

Eventually, Gourd et al. (1993) observed that, nearly 48 hours after inoculation, J<sub>2</sub>'s were seen penetrating to the roots near the root tip region in the soybean plants, but in this study, we found J<sub>2</sub>'s in the roots of muskmelon merely a day after inoculation, thereby showing the susceptibility of the plant to *M. incognita*. Soon after the invasion of J<sub>2</sub> inside the root tissues, it established a permanent feeding site in the vascular region of the root, where it becomes stationary and starts feeding. As a result, changes occur in the root's physiology, leading to the formation of specialized "Giant cells" around the nematode's head. As the nematode continues to grow, it will become "sausage-shaped" and molts again to become a third-stage juvenile (J<sub>3</sub>). Further development involves another molt, transforming the nematode into fourth stage juvenile (J<sub>4</sub>), which can be distinguished as either male or female, and the females remain sedentary at the feeding site for the rest of their lives.

After a fourth molt, the developing male leaves the root. Meanwhile, the female, after the fourth molt, continues to swell and becomes "pear-shaped". Adult females lay

approximately 250-400 eggs in an egg sac located outside the root tissues. These eggs hatch within a few days or remain dormant until a suitable host is found. These findings are in accordance with Taylor and Sasser (1978); Mukthar et al. (2017); and Kavitha et al. (2011).

## CONCLUSION

Understanding this life cycle is crucial for developing effective strategies to manage and control the root-knot nematode and mitigate its impact on agricultural crops. Further research into the molecular and physiological aspects of this interaction holds promise for developing innovative and sustainable solutions in agriculture. The knowledge obtained from this research can guide the formulation of precise approaches to address root-knot nematode (RKN) infestations in muskmelon crops, ultimately bolstering yields and guaranteeing top-notch produce for consumers. It is imperative to continue with additional investigations and foster collaborative endeavours to fine-tune these strategies and confront the enduring challenge posed by RKN to worldwide agriculture.

## ACKNOWLEDGMENTS

The authors are highly thankful to the "Other Backward Classes Cell" (OBC-Cell), University of Mysore, Mysuru, for providing financial support through fellowship. The authors are also thankful to the Department of Nematology, Tamil Nadu Agricultural University (TNAU), Coimbatore-03, Tamil Nadu, for the training program on Basic Nematological Techniques.

## CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest.

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