

## THE RICE ROOT-KNOT NEMATODE, MELOIDOGYNE GRAMINICOLA – LIFE CYCLE AND HISTOPATHOLOGY

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### Abstract

*Meloidogyne graminicola* (Golden and Birchfield) is a major pest of rice throughout the world due to its broad host range and ability of causing potential yield loss. The losses caused by *M. graminicola* may vary from negligible to heavy depending on the severity of disease. The main symptoms of root knot disease of rice are yellowing, dwarfing and gall formation on the roots of rice plants. The degree of symptom manifestation differs with time of infection, age of the plants and load of inoculum. The life cycle of rice root-knot nematode, *M. graminicola* in rice was studied under glasshouse conditions at Tamil Nadu Rice Research Institute, Aduthurai. The duration of life cycle ranged from 25- 28 days at ambient temperature  $28 \pm 2^\circ\text{C}$ . The adult females were smaller in size and laid eggs inside the root. Histopathological studies of the infected root revealed a severe dislocation of xylem and phloem vessels in the cortical region which resulted in the formation of giant cells.

**Keywords:** Life Cycle, Histopathology, *M. Graminicola*.

### Introduction

Rice grown in different environments is attacked by different nematode species like *Ditylenchus angustus*, *Meloidogynespp.Hirschmanniella* spp. *Aphelenchoides besseyi* and *Pratylenchus* spp. The rice root-knot nematode, (*Meloidogyne graminicola*) and rice root nematode (*Hirschmanniella oryzae*) are economically damaging genera of plant-parasitic nematodes attacking rice. Among

them rice root-knot nematode has gained considerable attention during the recent times because of its damage potential to rice particularly under water stress conditions (Tusharet *al.*, 2012). Severe infestations on rice were observed in the recent past in the Cauvery Delta Zone where direct seeded rice (DSR) has been widely practiced. They cause about 16-32% loss in grain yield due to incomplete filling of kernels. The seedlings exhibit profused galling on roots and depletion in vigour,

yellowing, stunting and curling of leaves. Water saving irrigation technologies such as aerobic rice, System of Rice Intensification (SRI) and direct seeded rice (DSR) are receiving renewed attention from researchers and farmers. Dry direct-seeded rice is a feasible alternative to conventional puddled transplanted rice with good potential to save water (Pankaj, *et al.*, 2010).

Root-knot nematode affected plants show depletion in vigor, stunted growth, chlorotic and curled leaves in nurseries and main field (Fig 1A). The nematode infection is characterized by the formation of small galls near the tips of the roots (Fig.1B,1C&1D). *M. graminicola* is an obligate parasite and a major pest of rice. Infective second stage juveniles select a point for entry into the root, usually in the meristematic zone. The juveniles cause disruption, hypertrophy and hyperplasia of cortical cells by intracellular migration and releasing oesophageal gland secretions.

Hence an investigation was done to study the life cycle and histopathology of rice root-knot nematode infesting rice in Cauvery Delta Zone.

### Materials and Methods

#### Life cycle of rice root-knot nematode in rice

Life cycle of root-knot nematode *M. graminicola* in rice was studied under glasshouse conditions at Tamil Nadu Rice Research Institute, Aduthurai. Twenty five days old rice seedlings of ADT 43 were planted in earthen pots filled with sterilized pot mixture. Rice root-knot nematode infested roots that were collected during field surveys served as source of inoculum. Egg masses were collected from the roots and eggs were allowed to hatch by incubating them in tap water for 3-4 days. Hatched infective second stage juveniles were inoculated to rice seedlings to study their life cycle in rice. Uninoculated rice seedlings were maintained as control.

Observations on the development of embryonic or pre parasitic stages *viz.*,

single celled, two celled, many celled, gastrula stages and first stage juvenile ( $J_1$ ) and post embryonic stages or parasitic stages *viz.*, second stage ( $J_2$ ), third stage ( $J_3$ ) and fourth stage juvenile ( $J_4$ ) and adult of *M. graminicola* were carried out to understand the life cycle and host parasite relationship. Studies were performed at ambient temperature of  $28 \pm 2^\circ\text{C}$ .

#### Histopathological studies of root knot nematode infected roots

Root samples of galled and healthy root tissues of rice were collected, gently washed with distilled water and stained with acid fuchsin lactophenol and sections were taken up using microtome by following the method suggested by Jonsen, (1962).

**1. Microtome:** Root samples were washed and fixed in FAA for minimum 12 hours (FAA: 10:50:5:35 proportion of formalin, alcohol, acetic acid and water).

**2. Dehydration:** After fixing the materials in FAA solution, they were washed with 50 per cent ethanol and then transferred to tertiary butyl alcohol series of 60, 70, 80, 90 and 100 per cent for an hour, followed by 12 hours in 100 per cent tertiary butyl alcohol (TBA).

**3. Infiltration with wax:** After the process of dehydration, the samples were then transferred to TBA with series of 2/3,  $\pm$  2/3, 1/3, 1/3, 1/2+1/2, 1/3+2/3 and absolute wax two times for 30-45 min in each series.

**4. Embedding:** Next to infiltration process, the material was embedded in wax with melting point of  $52-54^\circ\text{C}$  and this molten wax was poured into a paper boat, with inner side smeared with glycerin. The infiltrated pieces were placed in molten wax in the proper orientation. The blocks were cut in such a way that block that each block contained one section.

**5. Sectioning:** The blocks were mounted to microtome holder and sections were taken up with thickness of  $12\mu$  using spencers rotary microtome.

**6. Dewaxing and staining:** The dewaxing was done using xylene alcohol mixture. The slides containing sections were kept for half an hour in pure xylene, ethanol + xylene (1:1), 90, 70, 50 per cent ethanol. The slides were kept in saffranin solution for 12 hours, and subsequently transferred to 50, 70, 90 per cent ethanol for 10 min in each series, picric acid mixture. Then, the slides were transferred to 70 per cent alcohol for 2-3 min. Fast green solution was added over the sections and stain was drained with clove oil and washed with distilled water. Slides were transferred to alcohol; xylene mixture for 5 min and in pure xylene for 10 min.

**7. Mounting:** The sections were in neutral in synthetic mounting (DPX mountant) and air dried.

### Results and Discussion

#### Life cycle of rice root-knot nematode in rice

As the nematode developed in the egg, it molted to change from a first stage to a second stage juvenile ( $J_2$ ) which then hatched out from the egg (Fig.2A)  $J_2$  is the only infective stage that burrowed into the root, usually at or near the root tip (Fig 2B). The infective second stage juveniles of *M. graminicola* entered the rice roots within 24 hrs of inoculation and they oriented parallel to the longitudinal axis of the root. The nematode started feeding and became stationary. Due to continuous feeding the body size increases. During this period the tail of the nematode remained unchanged which showed spiked appearance (Fig 2C) at the perineal region. After 14-15 days of inoculation the developing females became typical flask shaped appearance which is called as pre adult stage (Fig 2D) and the posterior region of the body increased in width as the ovary increased in size due to egg production.

During feeding, normally the nematode releases enzymes and plant growth hormones into the root. This caused changes in the root's physiology, and "giant cells" were formed around the

nematode's head. Generally 5-7 giant cells develop and the nematode moves its head slightly to feed on these specialized cells. Nematodes examined after 20 days of inoculation were fully grown (Fig 2F) and the posterior end of the females lied in the middle portion of the cortex region and laid eggs in gelatinous matrix in the cortex very close to the epidermis. The duration of second, third, fourth and adult female stages lasted for 1-5, 6-8, 9-12 and 28 days respectively. Females laid about 250-300 eggs in an egg sac inside the root tissues (Plate 7H). The total life cycle including the preparasitic stage was 25-28 days (Table 2). Root tissues became enlarged to form a gall or "root-knot" around the nematode (Fig 2I). No gall formation was observed in the uninoculated plants. Female gets fertilized by the male (Fig 2G) and lays about 250-300 eggs in an egg sac inside the root tissues. Duration of different stages in the life cycle of rice-root knot nematode recorded in rice is given in table 1. Life cycle of root knot and cyst nematodes was described by McKenry and Roberts, (1985).

#### Histopathological studies of root knot nematode infected roots

Results of the histopathological studies of the nematode infected and healthy roots showed the formation of specialized feeding sites called "giant cells" which were the modification of procambial cells of the vascular region of rice roots. Section showed multinucleated giant cells with large vacuoles and dense cytoplasm. Xylem and phloem vessels were heavily dislocated in the infested root tissues. Hypertrophy and hyperplasia of cortical cells were noticed which contributed to the formation of root galls (Fig 3a). In the healthy uninfected roots in the uninoculated pots, no giant cell formation was observed and the xylem and phloem vessels were intact (Fig 3b).

Histopathological studies of the nematode infected root that showed the formation of specialized feeding sites called "giant cells" which were the modification of

procambial cells of the vascular region (Bird, 1979). Giant cells are multinucleated with large vacuoles which act as metabolic sink supplying nutrients for the developing female and throughout its parasitism. Similar observation was recorded by Dropkin (1969) in tomato.

**Conclusion**

Several management methods including physical, chemical, cultural, biological and host plant resistance are available to manage nematodes in rice ecosystems. An integrated approach rather than adopting a single method would help in an effective nematode management as each method has its own advantage for sustainable rice production.

From the study it is evident that rice root-knot nematode, *M. graminicola* is a great threat to the rice crop and it warrants a suitable nonchemical management strategy considering the economic importance of the crop.

**Table 1. Duration of different stages in the life cycle of rice-root knot nematode *Meloidogyne graminicola* (At ambient temperature 28± 2°C)**

Life stages	Duration (Days)
Second stage juvenile (J <sub>2</sub> )	1-5
Third stage juvenile (J <sub>3</sub> )	6-8
Fourth stage juvenile (J <sub>4</sub> )	9-12
Adult male	23
Adult female	26
Total life cycle	25-28

**Fig 1. Symptoms of rice root-knot nematode, *M. graminicola* infestation**



**A. *M. graminicola* infested field**



**B. *M. graminicola* infested rice plant**

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