



## Comparison of Migration, Penetration, Development, Reproduction and Histological Responses of Rice Root Knot Nematode, *Meloidogyne graminicola* between Susceptible (CO 47) and Moderately Resistant (ADT 45) Rice Varieties

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Comparison of migration, penetration, development, reproduction of *Meloidogyne graminicola* and histological responses between susceptible (CO 47) and resistant (ADT 45) rice varieties were made. In susceptible variety, second stage juveniles (J2) readily penetrated into the roots within 24h and developed into adult females in 20<sup>th</sup> day and started laying eggs on 23<sup>rd</sup> day. Comparatively fewer J2 penetrated in moderately resistant variety and developed slowly into adult on 28<sup>th</sup> day. Eggs and J2 of next generation were recorded on 30<sup>th</sup> and 32<sup>nd</sup> day after inoculation respectively. In susceptible roots cluster of giant cells and mature females were found in the vascular region in moderately resistant variety even though penetration of juveniles observed inside the root system.

**Key words:** Histological response; *Meloidogyne graminicola*; rice varieties.

Several factors are responsible for the low productivity of rice. Of these, availability of irrigation water, soil nutrient status and outbreak of insect pests and diseases are major constraints to higher productivity (Pimentel, 1983). The rice root-knot nematode *Meloidogyne graminicola* belonging to the family *Heteroderidae* is one of the most economically important nematodes affecting rice. It has been reported to cause significant yield loss of 20-50 per cent in many rice producing countries of India, Bangladesh, Philippines, Thailand, Vietnam, Cambodia and Indonesia (Arayarungarit, 1987; Prot *et al.*, 1994; Cuc and Prot, 1992; Soriano and Reversat, 2003 and Padgham *et al.*, 2004). Considering the increasing importance of *M. graminicola* it is programmed to compare the migration, penetration, development, reproduction and histological response of between susceptible and moderately resistant rice varieties.

### Materials and Methods

Sprouted seeds of susceptible (CO 47) and moderately resistant (ADT 45) rice varieties were seeded in earthen pots containing 1 kg soil. Five pots were used as replicates. Immediately after sowing, a nematode suspension containing 2000 active second-stage juveniles (J2) was inoculated into each pot and watered to field capacity.

In order to determine life- cycle, 10 seedlings infected with nematodes were sampled daily until the eggs and the J2 were detected by staining with acid fuchsin lactophenol using the method of Daykin and Hussey (1985). The stained roots were then

transferred into Petri dishes and root galls were cut and separated from the root systems. Each root gall was transferred onto a glass slide containing a few drops of glycerol solution (equal parts of glycerol and distilled water). To identify the different nematode developmental stages the different nematode developmental stages were separated from the root tissues with a needle and mounted on permanent slides for microscopic observation and photography.

For histological responses segments of moderately resistant and susceptible roots were sampled 45 days after inoculation of the nematode and fixed in TAF (Triethanolamine – 2ml, Formalin – 7ml and distilled water – 9ml) at 70°C for 24 h. The root segments were dehydrated through the ethyl alcohol series and embedded in paraffin wax for microtomy sectioning. Fixed root segments were then sectioned at 10 - 15µm, stained in safranin and fast green on gelatine coated glass slides and mounted in Dummar xylene according to Johansen's method (1940). Root sections of moderately resistant and susceptible varieties were observed at 45 days after inoculation (DAI).

### Results and Discussion

In CO47, the penetration of second stage juveniles occurred after 24h of nematode inoculation whereas in ADT45 it occurred on 2<sup>nd</sup> day (Table 1). Development of males occurred on 14<sup>th</sup> and 18<sup>th</sup> DAI in CO47 and ADT45 respectively. Adult female started to lay eggs on 23<sup>rd</sup> day in CO47 and 30<sup>th</sup> day in case of ADT45. The completion of life cycle from 2<sup>nd</sup> stage juvenile to second generation 2<sup>nd</sup> stage juvenile took place in m25 days in CO47 and 32 days in ADT45.

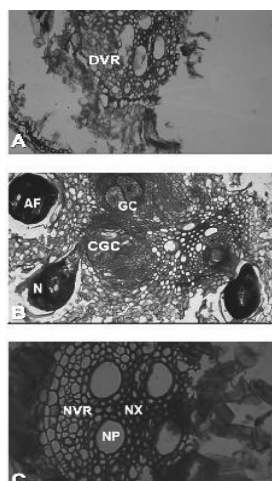
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**Table 1. Comparison of migration, penetration, development and reproduction of *M. graminicola* in rice varieties**

Particulars	Susceptible variety (CO 47)	Moderately resistant variety (ADT 45)
Penetration	At 24hrs	At 2 <sup>nd</sup> day
Second stage juveniles (J <sub>2</sub> )	On 3 <sup>rd</sup> day	On 5 <sup>th</sup> day
Third stage juvenile (J <sub>3</sub> )	On 7 <sup>th</sup> day	On 10 <sup>th</sup> day
All stages	On 12 <sup>th</sup> day	On 14 <sup>th</sup> day
Males	On 14 <sup>th</sup> day	On 18 <sup>th</sup> day
Root gall	On 17 <sup>th</sup> day	On 22 <sup>nd</sup> day
Females	On 20 <sup>th</sup> day	On 28 <sup>th</sup> day
Egg laying	On 23 <sup>rd</sup> day	On 30 <sup>th</sup> day
J in second generation	On 25 <sup>th</sup> day	On 32 <sup>rd</sup> day

**Histological responses of rice varieties to *M. graminicola***

In susceptible roots, cluster of giant cells, pre adult and several mature females were found in the vascular region. The formation of giant cells resulted in abnormal vascular region. It was observed that the females developed within the root and eggs were mainly laid in the cortex. Giant cells were observed around the heads of nematodes in the vascular region of the root, and not in the cortical region. A group of 4 – 9 giant cells was observed and these cells had thick walls and multinucleated dense cytoplasm while some cells were vacuolated. But in ADT45, the development of giant cells was not observed even though juveniles penetrated inside the root system and there was no alteration in the vascular region (Fig. 1).



A. Moderately resistant root B. Susceptible root C. Healthy root  
 DVR: Deformed Vascular Region by nematode feeding  
 AF: Adult Female GC: Giant Cell CGC: Complex Giant Cell  
 N: Nematode NVR: Normal Vascular NX: Normal xylem  
 NP: Normal Phloem

**Fig. 1. Comparison of histological responses of susceptible (CO 47) and moderately resistant (ADT 45) rice varieties to *M. graminicola***

In this study, the life cycle of *M. graminicola* was completed in 25 and 32 days respectively in susceptible and moderately resistant rice varieties

at 19–32°C. Several workers studied the life cycle of *M. graminicola* (Golden and Birchfield, 1968) in rice roots and reported differences in duration. Rao and Israel (1973) reported the life cycle as 26–51 days, depending on the season and Bridge and Page (1982) reported as 19 days from 22 to 29 °C. The development and duration of the life cycle of root knot nematodes (*Meloidogyne spp*) depended on the type of host and environmental factors. Due to the availability of optimal environmental conditions many tropical *Meloidogyne spp*. had a short life cycle with many generations (Rohini *et al.*, 1986). In comparison with susceptible variety the development of giant cells was not observed in moderately resistant variety. Williamson and Kumar (2006), recorded similar observations.

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