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Review

Global status of rice root-knot nematode, *Meloidogyne graminicola*

Tushar K. Dutta*, Ajoy K. Ganguly and Hari S. Gaur

Division of Nematology, Indian Agricultural Research Institute, New Delhi-110012, India.

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In the recent past, rice root-knot nematode, *Meloidogyne graminicola* has attained wide importance due to its potential to cause major damage in rice-wheat cropping system. It has become an emerging problem in the nurseries and upland rice along with its widespread occurrence in the deepwater and irrigated rice in the different countries of Southeast Asia. Second stage juveniles (J2s) penetrate the roots closely behind the root tip; migrate to vascular cylinder turning it into multinucleated giant cells, by endomitosis and cell hypertrophy characterized by hook shaped galls. This nematode is remarkably well adapted to flooded condition as it can survive in the aerenchymatous tissue of the graminaceous plant in flooded condition.

Key words: Continents, incidence, loss/damage, rice-wheat cropping sequence.

INTRODUCTION

A prime example of how a combination of agricultural, environmental, socioeconomic, and policy changes can affect the pest status of a plant-parasitic nematode in the tropics is illustrated by *M. graminicola* on rice in Southeast Asia. A combination of socioeconomic and environmental (climate) changes is responsible for increasing water shortages, not only increasing the cost of rice production but also severely limiting yields of rice, thus threatening food security.

The root-knot nematodes (RKN) are serious pests of vegetables, fruits, ornamentals and other dicotyledonous and some monocotyledonous plants. Their species, *Meloidogyne incognita*, *M. javanica* and *M. arenaria,* widespread in subtropics and tropics and *M. hapla,* found in sub-temperate and temperate regions, are serious pests of dicotyledonous crops although these occasionally infect cereals (Sasser, 1980; Sasser et al., 1984; Dasgupta and Gaur, 1986; Soriano and Reversat, 2003; Somasekhar and Prasad, 2009). *Meloidogyne graminicola* is known to infect and cause serious damage to cereals, especially rice, in many countries (Arayarungsarit, 1987; Bridge, 1990; Plowright and Bridge, 1990; Prot and Matias, 1995; Padgham et al., 2004; Pokharel et al., 2007). It is a serious problem in the nurseries and upland rice but has been recently found to be widespread in the deepwater and irrigated rice also, in many states of India (Prasad et al., 1985, 1886; Rao et al., 1986; MacGowan, 1989; Bridge et al., 1990; Jairajpuri and Baqri, 1991). The root-knot nematodes, *M. graminicola* and *M. triticoryzae,* infecting rice and wheat also cause serious losses to rice crops in some areas in north India (Gaur et al., 1993, 1996).

Meloidogyne graminicola Golden and Birchfield, is the most common RKN species infecting rice. In India, it is reported to cause 17-30% yield loss due to poorly filled kernels (MacGowan, 1989; Jain et al., 2007).

HOST RANGE

M. graminicola, M. naasi, M. oryzae, M. salasi, M. triticoryzae etc. generally prefer cereal hosts but can also infect some dicotyledonous plants (Birchfield, 1965; Yik and Birchfield, 1979; MacGowan and Langdon, 1989; Gaur and Sharma, 1999; Sabir and Gaur, 2005).

Tomato cv. Rutgers was an experimental host for most of the root-knot species, but did not allow multiplication of root-knot species attacking cereals, like *M. graminicola*, *M. oryzae* and *M. graminis* (Sasser and Triantaphyllou, 1977). However, *M. oryzae* multiplied well in tomato cv. Money Maker (Maas et al., 1978) in contrast to *M. graminicola* that did not multiply in any of the tomato cultivars tested (Manser, 1971). *M. graminicola* galled roots and reproduced at higher rates than *M. incognita* on

*Corresponding author. E-mail: nemaiari@gmail.com.

most of the *Trifolium* species evaluated. Although the two root-knot species varied in their ability to gall *Trifolium* species, the level of root galling indicated that most of the *Trifolium* species evaluated were susceptible to both root-knot nematode species (Windham and Pederson, 1992).

Aside from the rice plant, it also prefers *Alopecurus* sp., *Avena sativa*, *Beta vulgaris*, *Brachiaria mutica, Brassica juncea, B. oleraceae, Colocasia esculenta, Cyperus procerus, C. pulcherrimus, C. rotundus, Echinochloa colona, Eleusine indica, Fimbristylis miliacea, Fuirena* sp*., Glycine max, Lactuca sativa, Lycopersicon esculentum, Monochoria vaginalis, Panicum miliaceum, P. repens, Paspalum scrobiculatum, Pennisetum typhoides, Phaseolus vulgaris, Poa annua, Ranunculus sp., Saccharum officinarum, Sorghum bicolor, Sphaeranthus* sp*., Sphenoclea zeylanica, Spinacia oleracea, Triticum aestivum,* and *Vicia faba* (MacGowan, 1989; MacGowan and Langdon, 1989).

DISTRIBUTION

Rice RKN is widely distributed in the countries of S.E. Asia, Burma, Bangladesh, Laos, Thailand, Vietnam, India, China, the Philippines and USA both on upland and lowland deepwater rice (Pankaj et al., 2010).

Rice production, especially in the lowlands, has raised international concern since the traditional paddy production system consumes large amounts of water, and in many areas of Southeast Asia the water requirement is too high to sustain this type of rice production. Furthermore, competing demand for urban populations and industry impose legal restrictions in the use of water for agricultural purposes; thus, the amount of arable land available for the cultivation of lowland rice with its inherently high water demand is being reduced. In Asia, out of a total area of 79 mha of irrigated paddy rice, 17 mha may experience physical water scarcity and 22 mha economic water scarcities by 2025. Thus water saving rice production systems, such as direct wet seeding, intermittent irrigation, cultivation on raised beds, and the cultivation of aerobic rice varieties, are being developed and increasingly implemented. However, observations increasingly indicate that the large scale introduction of these techniques is favouring the development of high populations of *M. graminicola*, drastically increasing its economic significance (De Waele and Elsen, 2007).

M. graminicola was first described in 1965 from grasses and oats in Louisiana (Golden and Birchfield, 1965). It has since been found on rice mainly in South and Southeast Asia but also in South Africa, United States, Colombia, and Brazil.

M. graminicola is equally prevalent on upland (rainfed) or lowland (irrigated) as on deepwater rice. It causes swellings and galls throughout the root system. Infected root tips become swollen and hooked, a symptom characteristic of this nematode species. In upland conditions and shallow intermittently flooded land, *M. graminicola* is considered to be by far the most damaging *Meloidogyne* species on rice. In *M. graminicola*-infested upland rice fields, nematicide application resulted in a yield increase of 12–33% in Thailand (Arayarungsarit, 1987) and 28–87% in Indonesia (Netscher and Erlan, 1993), whereas under simulated upland conditions, yield losses from *M. graminicola* ranged from 20 to 80% (Plowright and Bridge, 1990). In *M. graminicola*-infested lowland rainfed rice, nematicide application resulted in a yield increase of 16–20% in Bangladesh (Padgham et al., 2004), and in simulations of intermittently flooded rice, yield losses from *M. graminicola* ranged from 11 to 73% (Soriano et al., 2000).

MORPHOLOGICAL FEATURES

Adult females appear to be pear-shaped to spheroid with elongated neck, which is usually embedded in root tissue. Their body does not transform into a cyst like structure. Females have six large unicellular rectal glands in the posterior part of the body, which excrete a gelatinous matrix to form an egg sac, in which many eggs are deposited. The stylet is mostly 9-18 µm long with three small, prominent, dorsally curved basal knobs. The esophageal glands overlap the anterior end of the intestine. The females have two ovaries that fill most of the swollen body cavity. The vulva is typically terminal with the anus, flush with or slightly raised from the body contour and surrounded by cuticular striae, which form a pattern of fine lines resembling human fingerprints called the perineal pattern.

Infective second stage juveniles are short (0.3-0.5 mm) and have a weak cephalic framework. The esophageal gland lobe overlaps the intestine ventrally. The tail tip tapers to a long, fine point with a long hyaline region.

ETIOLOGY

M. graminicola is a damaging parasite on upland, lowland and deepwater rice. It is well adapted to flooded conditions and can survive in waterlogged soil as eggs in eggmasses or as juveniles for long periods. Numbers of *M. graminicola* decline rapidly after 4 months but some egg masses can remain viable for at least 14 months in waterlogged soil. *M. graminicola* can also survive in soil flooded to a depth of 1 m for at least 5 months. It cannot invade rice in flooded conditions but quickly invades when infested soils are drained. It can survive in roots of infected plants. It prefers soil moisture of 32%. It develops best in moisture of 20 to 30% and soil dryness at rice tillering and panicle initiation. Its population increases with the growth of susceptible rice plants. The presence of relatively broad host range and many of the alternative vegetable crops that are grown during dry

Figure 1. Characteristic hook like galls stained with acid fuchsin showed numerous number of *M. graminicola* juveniles.

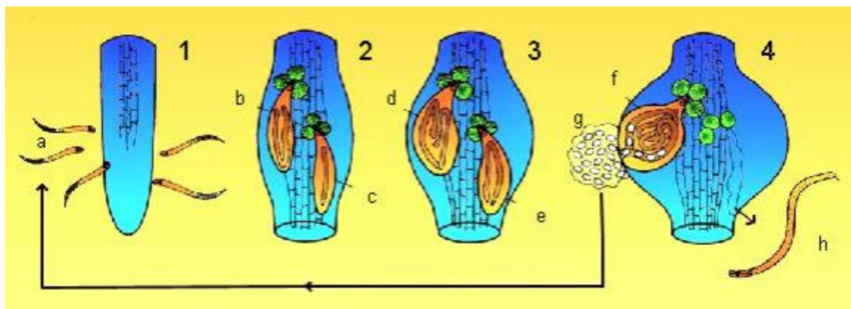

Figure 2. Life cycle of *M. graminicola.* Photograph has been borrowed from www.irri.org. Egg mass of *M. graminicola* is formed inside the root gall.

season are favorable for this nematode. A temperature of 22-29ºC is suitable for the prevalence of the nematode (Rao and Israel, 1973; Singh et al., 2003).

SYMPTOMS

1. Characteristic hook-like galls on roots (Figure 1).
2. Newly emerged leaves appear distorted and crinkled along the margins.
3. Stunting.
4. Chlorosis.
5. Heavily infected plants flower and mature early.
6. The roots of the host plants can be examined for hook-like galling. They can be stained to determine the presence and populations of *M. graminicola*.
7. The juveniles of *M. graminicola* can be extracted from the roots of the host plants.

BIOLOGY AND LIFE CYCLE

The rice root-knot nematode, *M. graminicola* is considered one of the limiting factors in rice production in all rice ecosystems. In upland rice, there is an estimated reduction of 2.6% in grain yield for every 1000 nematodes present around young seedlings. In irrigated rice, damage is caused in nurseries before transplanting or before flooding in the case of direct seeding. Experiments have shown that 4000 juveniles per plant can cause destruction of up to 72% of deepwater rice plants by drowning out. Statistically significant reduction in plant weight was recorded at the inoculum level of 2000 J2/kg soil (Sharma, 2001, Kanwar et al., 2008).

J2 penetrate the roots closely behind the root tip; migrate first towards the root tip, where the absence of differentiated endodermis allows them to enter the vascular cylinder followed by intercellular migration by mechanical and possibly enzymatic softening of the middle lamella. The parasites finally start feeding on three to ten cells, which are rapidly turned into multinucleated giant cells, by endomitosis and cell hypertrophy. The cells of the neighboring pericycle start to divide, giving rise to a typical hook like gall within 72 h. This particular nematode can reproduce through amphimixis as well as meiotic parthenogenesis completes its life cycle in 26-51 days in different periods of the year. In Bangladesh the nematode completes its life cycle by 19 days at 22-29ºC (Bridge and Page, 1982).

Following is the different steps undertaken by this particular nematode to complete its life cycle (Figures 2 and 3):

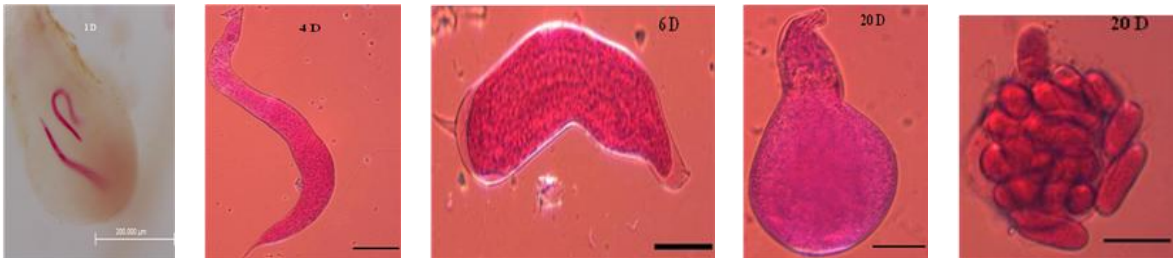

Figure 3. Different life cycle stages of *M. graminicola* at different days. 1 Day-second stage juvenile, 4 Day-third stage juvenile, 6 Day-fourth stage juvenile, 20 Day-adult female, 20 Day-eggs.

1. The larvae of the second stage (a) are attracted to the roots. They usually penetrate the roots closely behind the root tip. The larvae then migrate first towards the root tip, where the absence of differentiated endodermis allows them to enter the vascular cylinder. This migration happens intercellularly by mechanical and possibly enzymatic softening of the middle lamella. The parasites finally start feeding on three to ten cells, which are rapidly turned into multinucleated giant cells, by endomitosis and cell hypertrophy.
2. At the same time as the giant cells are formed, the cells of the neighboring pericycle start to divide, giving rise to a typical gall or root-knot. Inside the gall, a female (b) and a male (c) of the J3 larval stage are shown.
3. The gall continues to swell, while females (d) and males (e) are in their J4 stage.
4. During the last moult, the male (h) dramatically changes its shape, then leaves the root, and fertilizes the female (f) in the case of amphimictic species. However, parthenogenesis is often encountered in root-knot nematodes. The female lays its eggs in a gelatinous matrix (g) inside the root. From there, the larvae of the second stage (a) hatch and are attracted to roots. Depending on environmental conditions, this cycle is completed in one to two months. (www.Knowledgebank.irri.org/riceDoctor/Fact_Sheets/Pests/Nematodes.htm.)

MOLECULAR STUDIES

RKN are worldwide in distribution and morphologically and genetically diverse. Using *SSU rDNA* analysis, De Ley et al. (2002) placed *M. incognita* a mitotic parthenogenetic species in clade I and *M. graminicola* a meiotic parthenogenetic species in clade III. These species of RKN have different modes of reproduction and are evolutionarily distant from each other (Triantaphyllou, 1985). *Meloidogyne graminicola*, unlike other *Meloidogyne* spp., is remarkably well adapted to flooded conditions, enabling it to continue multiplying in the host tissues even when the roots are deep in water (De Waele and Elsen, 2007).

Nevertheless, little is known about the molecular mechanisms that make a particular plant a host or a non-host for a given plant parasitic nematode species. Some non-host plants react to invading nematodes with an active hypersensitivity response possibly indicating the presence of a resistance gene, but in other plants pre-formed nematicidal metabolites such as alkaloids, phenolics and sesquiterpenes as well as phytoalexins produced in response to invasion appear to play a role in nematode rejection. Non-host plants may lack the genes for susceptibility required for production and maintenance of feeding site. Development of fundamental studies to understand what causes a plant to be a non-host could contribute to the development of novel strategies to control plant parasitic nematodes based on disruption of nematode behaviour or adjustment of host response (Dutta et al., 2011).

MANAGEMENT

There are cultural, biological, physical, mechanical, use of resistant varieties and chemical control that are available for the rice root-knot nematode. For example, cultural control includes continuous flooding, raising the rice seedlings in flooded soils, and crop rotation. These practices will help prevent root invasion by the nematodes. Soil solarization, bare fallow period and planting cover crops such as sesame and cowpea has been reported to decrease nematodes. Rotation crop like marigold (*Tagetes sp.*) is also effective in lowering root knot nematode populations because of its nematicidal properties.

The options to control *M. graminicola* are still limited. Only continuous flooding appears to be effective (Kinh, 1982), although yield losses may be minimized when the rice crop is flooded early and kept flooded until a late stage of development (Soriano et al., 2000).

Crop rotation with non-host crops like sweet potato, cowpea, sesamum, castor. Sunflower, soybean, turnip, cauliflower, jute, mustard and chickpea, resistant varieties (TKM-6, Patna 6, Dumai, Ch 47, Hamsa) has been found effective in reducing *M. graminicola* infestation (Rao, 1985).

There are some IRRI cultivars, which are resistant against the nematode. Likewise, some related rice species such as some accessions of *Oryza* *longistaminata* Chev. et Roehr. and *O. glaberrima* Steud are also resistant (Soriano et al., 1998).

The biological control agents like *Trichoderma harzianum*, *T. virens*, *Catenaria anguillulae* have showed promising results against this nematode (Pathak and Kumar, 2003; Singh et al., 2007).

Several nematicidal compounds can be used as chemical control. They are volatile (fumigants) and nonvolatile nematicides applied as soil drenches and seedling root dips or seed soaks to reduce nematode populations. Seeds can be treated with EPN and carbofuran. The roots can be dipped in systemic chemicals such as oxamyl or fensulfothion, phorate, carbofuran, and DBCP. Telone (1,3-dichloropropene) can be injected into the soil before the crop is planted.

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