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Rice Root-Knot Nematode (*Meloidogyne graminicola*) an Emerging Problem

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A B S T R A C T

Of late, rice root-knot nematode, *Meloidogyne graminicola* has emerged as a major threat throughout the world due to its adoptable nature, broad host range and ability of causing potential yield loss. The losses caused by *M. graminicola* may vary from 16-80 %. The daignostic symptoms of root knot disease of rice are, stunted growth, yellowing, and gall formation on the roots of rice plants. The degree of symptom manifestation depends on several factors, *viz.*, time of infection, age of the plants and load of inoculums. Abiotic factors such as nutrition, temperature, soil type, moisture, etc., play on important role on nematode population. Indiscriminate use of chemicals for managing this disease results in the development of pathogen resistance and risk to the environment. Ecofriendly approaches such as, removal of host weeds, flooding of fields, summer ploughing, organic amendments, and use of effective biocontrol agents and resistant cultivars has some advantage in managing the disease.

Introduction

Meloidogyne spp., the root-knot nematodes are obligate endo-parasites and perfect examples of highly adapted root parasitism. They are one of the major constraints in the production of field and plantation crops throughout the tropical, subtropical and temperate countries. These nematodes enjoy a guaranteed continuous supply of food and water from the host and protection within the gall for the females and their progeny. The damage to plants is due largely to the disruption of vascular tissues and extensive hypertrophy and hyperplasia of root cells. Besides the knotted roots, an infected plant shows poor growth, unthriftiness and general wilt symptoms the damage is aggravated by the parasites interaction with other pathogens such as fungi and bacteria (Siddiqi, 1986).

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 (Dutta *et al.,* 2012).

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 (McGowan, 1989; Jain *et al.,* 2007).

Meloidogyne graminicola is the major species attacking both upland (rainfed) and lowland (irrigated) rice. It is well adapted to flooded conditions and yield losses of up to 87% have been reported (Lilley *et al.,* 2011; Netscher and Erlan, 1993; Padgham *et al.,* 2004;Soriano *et al.,* 2000;Tandingan *et al.,* 1996). Its short life cycle and wide host range, including many weeds that are common in rice fields, make this species difficult to control (De Waele and Elsen, 2007). An effort has been made by scanning available literature to gather all the information with regard to various aspects of *Meloidogyne graminicola*. The overall presentation appeared as a comprehensive monograph on rice root knot nematode, probably, after 50 years of discovery of rice root knot nematodes from the world. Hence, utmost care has been taken, while scanning the literature published so far. However, there are chances of missing few references. Omissions, if any, are accidental and not intentional.

History and background

Root-knot nematode (*M. graminicola)* was found infecting grasses-*Echinochloa colonum, Poa annua, Alopecurus carolinianus, Eleusine indica* and oats in USA during 1965 (Golden and Birchfield, 1965). Later, this nematode was described as *Meloidogyne graminicola* (Golden and Birchfield, 1968). This was followed by several reports of its association with rice in many countries. In India, *M. graminicola* is the dominant species infecting rice. *M. triticoryzae* infecting both rice and wheat including some monocot weeds is also reported from India (Gaur *et al.,* 1993) and its occurrence is restricted to a few areas. The root-knot nematode is making its importance felt in almost all the rice growing areas.

Recent observations on the susceptibility of main crop to root-knot nematode in the rice based cropping system such as wheat (Chandel *et al.,* 2002), onion (Gregon *et al.,* 2002; Ravindra *et al.,* 2013) and banana (Reversat and Soriano, 2002) contribute to the accentuation of the problem. In Philippines, economic reasons and the decrease in water supply have induced the large scale adoption of direct wet seeding, chemical weed control and intermittent irrigation that favour the development of *M. graminicola* and have drastically increased its economic significance. Kreye *et al.,* (2009 a,b) observed that root-knot nematode was one of the important factors responsible for the poor plant growth and yield failure in aerobic rice in Philippines. Rice root-knot nematode appeared in devastating form in parts of major rice growing areas of Shimoga, (Karnataka, India) Krishnappa *et al.,* (2001).

Subsequently, sudden outbreak of *M. graminicola* infestation in 1500 ha area in Mandya (Karnataka, India) during *kharif*, 2001, stands as an example for our limited understanding of this nematode (Prasad *et al.*,2001). Sehgal *et al.,* (2012) reported that the severe outbreak of rice root-knot nematode from Shimoga, Karnataka. Ravindra *et al.,* (2014a) found incidence of root-knot nematode from all major rice growing districts of Karnataka but level of incidence differed from region to region and developed Digital map of Distribution of rice root-knot nematode in Karnataka.

Distribution of *M. graminicola*

M. graminicola is distributed in the countries of S.E. Asia, Burma, Bangladesh, Laos, Thailand, Vietnam, India, China, Philippines, Nepal and USA. *M. oryzae* has been found in Surinam on irrigated rice, *M. incognita* in Costa Rica, Cuba, Egypt, Ivory Coast, Nigeria, South Africa and Japan, *M. javanica* in Brazil, Egypt, Comoro Islands, Nigeria and Ivory Coast, *M. arenaria* in Nigeria, Egypt and South Africa and *M. salasi* in Costa Rica and Panama on upland rice (Bridge *et al.,* 1990). Pakistan (Munir and Bridge, 2003) Jammu and Kashmir (Singh *et al.,* 2007) In India, *M. graminicola* has been found infecting rice in Assam, Andhra Pradesh, Karnataka, West Bengal, Orissa, Kerala, Tripura and Madhya Pradesh (Prasad *et al.,* 1987). Root-knot nematode, *M. graminicola* is a serious pest of upland rice and nurseries world over in well-drained soils (Rao *et al.,* 1986b). The nematode was reported on irrigated rice in Andhra Pradesh (Sharma and Prasad, 1995) and Karnataka (Krishnappa *et al.,* 2001; Prasad *et al.,* 2001). The nematode can infect and multiply on semi-deep (Prasad *et al.,* 1985) or deepwater rice also (Bridge and Page, 1985). Windham and Golden (1988) from Mississippi. Occurrence of *M. triticoryzae* is reported from Delhi, Uttar Pradesh and Haryana (Gaur *et al.,* 1993). Catling and Islam (1999) in Asian deepwater rice. Nagaraju *et al.,* (2002) from K. R. Pet (Mandya) and K. R. Nagar (Mysore) taluks of Karnataka. (Ravindra *et al.,* 2014b) major rice growing districts of Karnataka. Narasimhamurthy *et al.,* (2015) observed severe incidence in Shimoga and parts of Davanagere districts of Karnataka.

Host range

M. graminicola has a wide host range with rice being a major economically important host. It was initially found on barnyard grass, *Echinochloa colonum* (Golden and Birchfield, 1965). Subsequently, it was found that it readily attacks several grasses, bush bean, oats (Golden and Birchfield, 1965), *Ranunculus pusillus*, *Cyperus compressus* L. (Yik and Birchfield, 1979), *Panicum miliaceum* L., *Pennisetum typhoides* (Burm. F) Stapf and C.E. Hubb and *Glycine max* (L.) Merr (Roy, 1978), *Echinochloa crusgalli*, *E. colona*, *Eleusine indica*, *Paspalum sanguinola*, *Eclipta alba*, *Grangea madraspatensis*, *Phyllanthus urinaria*, *Fimbristylis miliacea*, *Blumea sp*., *Vandellia sp.*,*Jussieua repens*,*Andropogon sp.,* chillies, tomato, wheat, Panicum spp. (Rao *et al.,* 1970), *Cyperus deformis* (Bajaj and Dabur, 2000), Banana (Reversat and Soriano, 2002) and onion (Gregon *et al.,* 2002; Ravindra *et al.,* 2013). *M. graminicola*, 8 of which are reported as new hosts of the nematode (*Digitaria sanguinalis*, *Bothriochloa intermedia* (Bothriochloa bladhii), *Physalis minima*, *Brachiaria ramosa*, *Alternanthera sessilis*, *Agropyron repens* (*Elymus repen*), *Dactyloctenium aegyptium* and *Sporobolus diander).* In the majority of the weeds, the female nematode concealed their egg masses within the galls, although some weeds, such as *Echinochloa colonum* (*E. colona*), *Cyperus rotundus*, *Physalis minima* and *S. diander*, had small egg masses on the root gall. In

Ageratum conyzoides, heavy root galling and production of eggs in the galls were observed (Khan, *et al.,* 2004). Anamika *et al.,* (2011) assess the disease incidence and intensity of root-knot disease on rice and vegetable crops in 21 districts of Uttar Pradesh (India).Fifteen different monocotyledons and dicotyledons weeds *viz,Digitaria longifolia, Hydrilla spp., Digitaria cingulata, Eragrostis unioloides, Cynotis culculata, Elucina indica, Echinochloa crusgalli, Echinochloa colonum, Cida acuta etc.,* infected by *M. graminicola* were reported from different rice growing districts Karnataka (Ravindra *et al.,* 2015)

Symptoms of damage

The rice crop in nursery-beds and main field appears uneven with patches of yellowish, stunted plants. The leaf size is reduced, tillering is poor and earhead emergence is delayed. In case of heavy infestation, no earheads may be produced. The earheads have poorly filled or no grains.

The crop in infested patches dries early during moisture stress. The symptoms produced due to the infestation of *M. graminicola* are manifested in the form of characteristic terminal hook or typical ring like spindle or bead/nodule shaped galls on the roots. The thicker roots of transplanted crop may appear irregularly thickened with no apparent cortical swellings but with stele swollen at various places (Plate 1).

Affected plants show depletion in vigour, yellowing of leaves and curling along the midribs. The second stage juveniles cause disruption and hypertrophy of cortical cells due to their migration, movement and salivary secretions. Hyperplasia of proto phloem and abnormal xylem proliferation cause swelling of stele at the site of nematode establishment (Jene and Rao, 1973). High initial population of *M. graminicola* causes seedling wilt along with severe reduction in growthparameters, whereas, low population cause only reduction ingrowth parameters (Plowright and Bridge., 1990).

Characteristic hook like galls on roots, newly emerged leaves appear distorted and crinkled along the margins, stunting, chlorosis, heavily infected plants flower and mature early, (Dutta *et al.,* 2012).

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 inoculums (Vinod *et al.,* 2014). The main symptoms caused by *Meloidogyne graminicola* are yellowing, stunting and gallformation on the roots of rice plants. The degree of symptom manifestation varies with inoculums load, time of infection, ageof the plants, *etc*.

Ravindra *et al.,* (2013) reported fifteen different galling patterns from Shimoga, Shikaripur, Bhadravati, Hosnagara, Sorab and Sagar taluks of Shimoga district. divergence from the typical symptom of galls at the root terminal *viz.,* bulbous galls at the mid of the roots, bunch of galls, superficial galls, discreet multiple galls, bulbous galls at the branching of roots were observed along with the typical symptoms of *M. graminicola* (Plate 2). As the samples collected were analyzed from different farmers' field it may be the sign of different species of root-knot nematodes infecting rice or indication of presence of different *M. graminicola* biotypes.

Biology and life cycle

M. graminicola males are capable of reproducing by amphimixis and meiotic parthenogenesis. About 200 – 500 unembryonated or partially embryonated eggs

are deposited in a gelatinous egg matrix. After 4-6 days of embryogenesis a first stage juvenile (J_I) is produced which moults in 2-3 days into a second stage juvenile (J_2). The infective J2 is ready to hatch out of egg under favourable environmental conditions, *i.e.*, temperature 20-35^{o}C and moisture. The rate of hatch is slow and extends over several weeks. This could be due to different time of laying of eggs by the females of different ages present in a cluster, and/or due to the occurrence in a certain proportion of eggs which do not hatch immediately. A certain proportion of the eggs may not hatch unless stimulated by an extrinsic factor like the host root diffusate. The unhatched J_2 can survive inside the egg for several weeks in relatively dry condition and absence of host. The hatched J_2 finds the root and enters it generally in the elongation zone and induces formation of syncytium and gall as typical of root-knot nematodes. The J_2 swells during 3-4 days after infecting and moults into 13 (spike-tail stage), J_4 and adult male or female stages. The 13 and J4 do not have functional stylet, hence do not feed and are enclosed in a cuticular sheath from the preceding J_2 stage. The adult males can be seen 13-15 days after inoculation and freshly laid eggs can be seen 3-4 days thereafter. Thus, the life cycle from egg to egg takes 25-28 days on both wheat and rice under optimum temperature conditions of 27-30^{o}C. During winter the life cycle on wheat is prolonged up to 65 days at low temperature.

M. graminicola is a meiotic parthenogen, with ahaploid chromosome number of 18.*M. graminicola* completes its life cycle in 26-51 days in different periods of the year (Rao and Israel (1973). Bridge and Page (1985) however, reported that the life cycle of *M.graminicola* was completed in 19 days at an ambient temperature of 22-29ºC. They further noted that the generation time of *M. graminicola* could be as little as 13 days at an ambient temperature of 25-35º C. Khan *et al.*,(1995) reported that females laid eggs 20 day after inoculation of J2 of *M.graminicola.* Yik and Birchfield (1977) studied on life cycle of *Meloidogyne graminicola* on *Echinochloa colonum* under laboratory condition. The infective second stage juveniles requires 16 days at 26°C for converting into matured egg laying female and egg to second stage juvenile was about 8-11 days at 26°C and female laid an average of 260-325 eggs in glass house and field samples. Halbrendt (1997) reported that *M. graminicola* can complete its life cycle in as little as 15-19 days due to higher production rate. Dabur *et al.,* (2004) reported that life cycle of this nematode was completed in 24 days. They reported that adult male and females were observed on day 10 and egg laying and release of juveniles were first observed on day 20 and 24, respectively. Singh *et al.,* (2006) reported that life cycle of *M.graminicola* required 15-20 days to complete its life cycle in rice during different months in eastern Uttar Pradesh condition where temperature usually ranges between 22-40º C (Table 1).

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 (Dutta *et al.*, 2012).

Survival Strategy

Survival of *M. graminicola* is more in moist and wet soil than in air dried soil. A few loosely coiled inactive J_2 without shrinkage can be extracted from wet soil and could be reactivated by aeration (few can survive by anoxybiosis). However, bioassay indicated the presence of much higher population density than the number of J_2 extracted. This indicates that these nematodes survive mainly in the unhatched egg stage. The rate of decrease of population density was lower in natural field conditions than in laboratory. The J_2 are sensitive to desiccation. The hatching of J_2 from egg is inhibited both in too wet and too dry soil. A certain proportion of eggs appear to be undergoing diapause and become dependent for hatching upon the presence of host root diffusate. This explains the sudden resurgence of these nematodes even in fields where rice has not been grown for a few years. (Gaur, 2003)

Pokharel (2004) reported that nematode-induced rice yield reduction was low when plots were supplied with nitrogen and phosphorus as compared to control plots (no fertilizer or compost). In contradict, Rao and Israel (1971a) found that addition of nitrogen up to 40 kg/ha to the soil resulted in increased reproduction of *M. graminicola*. Application of additional phosphorus either alone or in combination with nitrogen also favoured nematode development. Rao and Israel (1972a) reported maximum hatching of eggs of *M. graminicola* in water at 25°C and 30°C. At 15°C and 35°C hatching was reduced and at 20°C it was slightly less than that at 25°C. Larval populations of *M. graminicola* in soil were large during December to February when soil temperatures were 20.9°C or less. Populations were small in March, July and August and very small in April, May and June when the soil temperature was 31°C. Maximum galls on rice roots were found during January to March and egg masses during February to March. Soil temperatures of 23.5°C or less were found most favorable for gall formation Rao and Israel (1971b). Larval invasion was greatest in soils at 32% moisture content; development and egg mass production were greatest at 20 to 30% soil moisture. Greatest larval invasion may occur at pH 3.5 but pH usually does not affect invasion, growth or development of the nematode to any significant extent In upland soil, which was well-drained and had 74-75% sand, larvae were observed up to a depth of 22-28 cm in nursery soil and 22 cm under transplanted crops Rao and Israel (1972a). In poorly drained lowland soil, larvae were observed up to a depth of 18 cm in both nursery and transplanted areas. In lowland soils the pore space was less and moisture content high so that the rice roots spread more laterally and nematode populations were greater at a depth of 2-6 cm compared with maximum density observed at a depth of 4-12 cm in upland soils. Coarse and medium soils with particles above 0.053 mm in diameter and sandy soils allowed free movement of infective larvae and invasion into roots of the rice plant. Clayey soils were less suitable tom nematode infection. With an increase in the sand content of the test soils, there was an increase in root growth, root-knot development and egg mass production by the nematode, the relationship between the sand content and the activity of the nematode was linear. Sandy or loamy, laterite soils or recent alluvial soils (in which the available soil nutrients range from moderate to low and

water holding capacity is low) favour development of the nematode Rao and Israel (1972b). Sandy or loamy, laterite soils or recent alluvial soils (in which the available soil nutrients range from moderate to low and water holding capacity is low) favour development of the nematode. It has been observed that waterlogged condition in the direct seeded rice or transplanted crop had no detrimental effects on the survival of the endoparasitic stages (Prasad *et al.,* 1985). Temperature of 22-29°C was found to be suitable for the prevalence of the nematode (Rao and Israel, 1973). Factors such as nutritional deficiencies, poor drainage, and soil-borne diseases can conceal the presence of nematodes. Soriano *et al.,* (2000) found greater damage to rice varieties in sandy soils than in clay soil. Population density of *M. triticoryzae* declined in puddled soil. Puddling reduced the bulk density of soil and decreased the hydraulic conductivity in the upper layers but not in the deeper layers where soil aeration was reduced due to high moisture levels retained in the puddled soil. The invasion of the roots by the second-generation infective juveniles was reduced. The population density of the root-knot nematodes was higher in the non-puddled soil especially in unsubmerged condition compared to puddled and submerged soil. However, where the seedlings were already infected before transplanting and submergence, the nematode could survive well and reproduce within the aerenchyma of the root (Chandel *et al.,* 2002). Sivakrishna *et al.,* (2013) reported that the nematode damage was significantly higher in unflooded condition compared to the flooded condition at both ambient (30-40°C) and at high (40-45°C) temperature.

Jagadeesh *et al.,* (2013a) reported that application of ammonia-based nitrogen fertiliser to the rice nursery bed may interfere with nematode attraction and thus reduce invasion, and the application of chemical nitrification inhibitors to rice nursery beds may decrease nematode invasion.

Jagadeesh*et al.,* (2013) reported that Roots supplied with a 100-fold lower supply of calcium nitrate (0.1 mM Ca(NO3)2) showed a higher level of nematode infection as measured by the gall index. Plants supplied with 2.85 mM of Ca (NO3)2 were more infected compared with the same dose of ammonium nitrate (NH4NO3) or ammonium chloride (NH4Cl). ^{15}NO3-influx studies showed significantly smaller uptake of nitrate in nematode-infected roots when compared with non-infected control plant.

Ravindra *et al.,* (2014c) reported that at pH range between 6.5 to <8.5 in the districts viz., Davanagere, Dakshina Kannada, Udapi, Uttar Kannada, Mysore, Kodagu and Haveri having moderate infection of *M. graminicola* with root-knot index of 3.0.

The survey on rice root-knot nematode in major rice growing district of Karnataka revealed that all the districts are infected with rice root-knot nematodes; however, there level of incidence differs in different districts. Among the districts, more severe incidence of rice root-knot nematode was observed with root knot index of 5 in Shimoga and 4 in Chickmagalure, Mandya and Hassan districts.

Moderate infection was noticed in districts viz., Davanagere, Dakshina Kannada, Udupi, Uttar Kannada, Mysore, Kodagu and Haveri with root knot index of 3, whereas, the Northern districts of Karnataka shows least root-knot index of 1, where the type of soil is black or vertisols. In all the rice growing districts, the organic content varied from <0.5 to >0.5. With regard to organic carbon content, no correlation was observed whether it was <0.5 or >0.5 as the same tendency of root-knot index and population levels were noticed in the districts where gall indices

varied from 3 to 5 and population levels. Ravindra *et al.,* (2015)

Host-parasite relationship

The root-knot nematode, *M. graminicola* is an obligate parasite and a major pest of rice. Infective second stage juveniles of *M. graminicola* 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. The nematode incites development of 5-8 giant or transfer cells in phloem. Around the giant cells abnormal xylem proliferation occurs that causes swelling in stelar tissue. Hypertrophy of cortical cells around the sites of establishment of the nematode is responsible for the formation of galls or root-knots. Inorganic nitrogen application to infected crop gives a temporary greenish appearance and the plants turn yellow within a week after application, due to the inability of roots to intake and transport the nutrients. Infection by the nematode results in reduction of N, P, K and increase in total sugars, amino nitrogen and DNA in plants. Increase in RNA in shoots and roots to an extent of 20 and 80%, respectively were recorded due to excessive hyperplasia and hypertrophy and inhibition of protein metabolism (Rao *et al.,* 1986c). The larval migration in cortex and establishment of giant cells in stele takes about two days in susceptible rice cultivars TN-1 and TN 4 while it takes about 12 days in resistant cultivar TKM 9 (Senthilkumar *et al.,* 2007). Rao and Israel (1972c) observed a higher rate of reproduction of *M. graminicola* at low levels of inoculums possibly due to the abundance of food, lack of competition and the ability of the host to support the population. The low rate of reproduction obtained at high levels of inoculum is considered to be due to crowding. The growth and development of the rice root-knot nematode population is thus dependent on its population density.

In deepwater rice, root-knot nematode infected seedlings remain stunted, unable to grow above flood water and perish due to continuous submergence. In Surinam, glasshouse trials revealed that the rice yield was 15% lower when *M. oryzae* inoculated in flooded soil and 9% less when nematode was inoculated in soil without standing water in comparison to nematode-free pots (Segeren and Bekker, 1985). Mishra and Mohanty (2007) observed an increase in phenolics by 28-104%, phenylalanine ammonia lyase by 16-35%, tyrosine ammonia lyase by 9-54%, decrease in amino acid tyrosine by 2-36% and amino acid tryptophan by 14-28% in rice cultivars Annapurna, Manika and Ramakrishna and suggested that these reactions could be used in rating of the resistance of cultivars to *M. graminicola.* Shrestha *et al.,* (2007) detected a total of six significant or putative QTLs for root-knot nematode tolerance and observed that the partial resistance to nematode establishment was related to nematode tolerance. They opined that it may be possible to breed plants with greater tolerance. Singh *et al.,* (2006) demonstrated the relationship between the biomass of *M. graminicola* developing in rice roots and the expression of disease symptoms. The biomass of invading second stage juveniles (0.09 μg) increased to 33μg on day 16 when adult females were in advanced egg laying stage, with an increase of approximately 360-fold. Initiation of leaf yellowing was related to the ratio between total nematode and total root biomass of rice seedlings. Plants with nematode root biomass ratios above 1: 161 did not show any symptom while those with ratios between 1: 138 and 1: 121 exhibited yellowing. Plants with nematode-to-root biomass ratios between 1: 115 and 1: 60 showed moderate stunting while those with ratios between 1: 43 and 1:

20 exhibited severe stunting. The wilting symptoms occurred at or below 1: 14 nematode-to-root biomass ratio. The yellowing of leaves in seedlings inoculated with graded inocula was expressed when the nematode-to-root biomass ratios reached to 1: 136 on day 15 at 1000 J_2, 1: 138 on day 9 at 3000 J_2, 1: 134 on day 7 at 6000 J_2 and 1: 129 on day 5 at 9000 J_2 per pot. In rice nurseries, seedlings showing moderate stunting, severe stunting, wilting and wilting with single gall were recorded at nematode-to-root biomass ratios of 1: 92, 1: 20, 1: 12 and 1: 14, respectively. In severely stunted transplanted rice, the nematode-to-root biomass ratio ranged from 1: 84 to 1: 75.

Interaction with other organisms and disease complexes

M. graminicola infestation causes reduction in phenols in the shoots and roots and it is thought to be the reason for greater susceptibility of nematode infected plants to rice blast pathogen, *Pyricularia oryzae* (Israel *et al.*, 1963) and root fungus, *Fusarium moniliformae* (Hazarika, 2001).

Effect of environmental factors

Rao and Israel (1972a) reported maximum hatching of eggs of *M. graminicola* in water at 25°C and 30°C. At 15°C and 35°C hatching was reduced and at 20°C it was slightly less than that at 25°C. Larval populations of *M. graminicola* in soil were large during December to February when soil temperatures were 20.9°C or less. Populations were small in March, July and August and very small in April, May and June when the soil temperature was 31°C. Maximum galls on rice roots were found during January to March and egg masses during February to March. Soil temperatures of 23.5°C or less were found most favorable for gall formation (Rao and Israel, 1971b). Larval invasion was greatest in soils at 32% moisture content; development and egg mass production were greatest at 20 to 30% soil moisture. Greatest larval invasion may occur at pH 3.5 but pH usually does not affect invasion, growth or development of the nematode to any significant extent. Drought conditions at the tillering stage and at both tillering and flowering stages favoured the development and reproduction of the nematode. Addition of nitrogen up to 40 kg/ha to the soil resulted in increased reproduction. Application of additional phosphorus either alone or in combination with nitrogen also favoured nematode development (Rao and Israel, 1971a). In upland soil, which was well-drained and had 74-75% sand, larvae were observed up to a depth of 22-28 cm in nursery soil and 22 cm under transplanted crops (Rao and Israel, 1972b). In poorly drained lowland soil, larvae were observed up to a depth of 18 cm in both nursery and transplanted areas. In lowland soils the pore space was less and moisture content high so that the rice roots spread more laterally and nematode populations were greater at a depth of 2-6 cm compared with maximum density observed at a depth of 4-12 cm in upland soils. Coarse and medium soils with particles above 0.053 mm in diameter and sandy soils allowed free movement of infective larvae and invasion into roots of the rice plant. Clayey soils were less suitable to nematode infection. With an increase in the sand content of the test soils, there was an increase in root growth, root-knot development and egg mass production by the nematode, the relationship between the sand content and the activity of the nematode was linear (Rao and Israel 1972d). Sandy or loamy, laterite soils or recent alluvial soils (in which the available soil nutrients range from moderate to low and water holding capacity is low) favour development of the nematode. It has been observed that waterlogged condition in the direct seeded rice or transplanted crop had no detrimental effects on the survival of

the endoparasitic stages (Prasad *et al.,* 1985). Temperature of 22-29°C was found to be suitable for the prevalence of the nematode (Rao and Israel, 1973). Factors such as nutritional deficiencies, poor drainage, and soil-borne diseases can conceal the presence of nematodes.

Population density of *M. triticoryzae* declined in puddled soil. Puddling reduced the bulk density of soil and decreased the hydraulic conductivity in the upper layers but not in the deeper layers where soil aeration was reduced due to high moisture levels retained in the puddled soil. The invasion of the roots by the second-generation infective juveniles was reduced.

The population density of the root-knot nematodes was higher in the non-puddled soil especially in unsubmerged condition compared to puddled and submerged soil. However, where the seedlings were already infected before transplanting and submergence, the nematode could survive well and reproduce within the aerenchyma of the root (Chandel *et al.,* 2002)

Yield losses

The crop loss is a function of population density and other crop growth conditions However, their occurrence is sporadic and localised. Golden and Birchfield (1965) for the first time recorded and described *M. graminicola* from the roots of barnyard grass, *Echinochloa colonum* in Louisiana. This nematode species is a common parasite of rice in many parts of world including India, Thailand, Bangladesh, Nepal, Philippines and Laos.

Over 31 rice cultivars were found infected in Louisiana (Golden and Birchfield 1968). It is now a C-rated pest in California though it was first reported on grasses. It is causing typical root gall in entire rice growing areas of Thailand and creates problems in nursery seedbed conditions (Buangsuwon *et al.,* 1971).

On upland rice, *M. graminicola* causes 16-32% loss in grain yield due to incomplete filling of kernels (Biswas and Rao, 1971; Chakrabarti *et al.,* 1971; Rao and Biswas, 1973). With increase in the inoculum given to 10 days old plants of cv. IR 8 by one egg mass, the corresponding reduction in grain yield was computed as 2.6% (Rao and Biswas, 1973). The threshold level to cause 10% loss is 120, 250 and 600 eggs/plant at 10, 30 and 60 days age of plants in direct seeded rice (Rao and Biswas, 1973).In India, outbreak of *M. graminicola* infestation in kharif rice has been witnessed in around 800 ha in Mandya district of Karnataka (Prasad *et al.,* 2001) and many parts of India like West Bengal, Orissa, Assam, UP, Himachal Pradesh, etc.; the emergence of this nematode problem in rice nursery has been encountered.A negative impact of *M. graminicola* on growth and yield of lowland rainfed rice in Bangladesh and aerobic upland rice in Nepal and India have been reported. In *M. graminicola* infested upland rice fields, nematicide application resulted in a yield increase of 12% to 33% in Thailand (Arayarungsarit, 1987) and 28% to 87% in Indonesia (Netscher and Erlan 1993) whereas, under simulated upland conditions, yield losses due to *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% to 20% in Bangladesh (Padgham *et al.,* 2004) compared to no nematicide treated field, and in simulations of intermittently flooded rice, yield losses from *M. graminicola* ranged from 11% to 73% (Soriano *et al.,* 2000). The impact of *M. graminicola* on rice yield has been well established, with yield losses up to 20% to 90%.

Detection and Diagnosis

During about 7 months in the year, the normal practice of Cobb's sieving and Bearmann funnel technique fails to detect these nematodes or gives a gross underestimate of the population density. Even wetting of the soil sample for a week does not help. However, a bioassay by sowing rice or wheat seed in the soil sample and observation of the root after 3-4 weeks to count the number of galls has been found to be a more reliable method to detect and estimate infestation levels in soil.

Scoring of RKI

Daignosis of *M. graminicola*

The species of root-knot nematode found on rice has been generally considered to be *M. graminicola*, based largely on the symptoms (hook-like galls produced on rice roots) and the ability of this species to cause infection in lowland rice. Accurate identification of nematodes is important and is central to understanding the host-parasite relationships and implementing appropriate management options. Traditional methods of nematode identification for root-knot nematode are based on morphology, perineal patterns (Chitwood, 1949; Eisenback *et al.*, 1981; van der Beek *et al.*, 1998) and a differential host range test (Sasser and Triantaphyllou, 1977). The perineal patterns alone could not be used to confirm the identity of the tested root-knot nematode (Plate 3). However, these methods are not completely reliable and can be time consuming (Eshenshade and Trintaphyllou, 1990). Esterase phenotype is considered to be a useful taxonomic character, but requires adult females at a specific developmental stage for accurate diagnosis (Eshenshade and Trintaphyllou, 1990). DNA sequences of the internal transcribed spacer (ITS) region of rRNA genes have been used successfully to identify species of nematodes (Zijlstra *et al.*, 1995, 1997; Hugall *et al.*, 1999; Powers, 2004). While PCR-RFLP approaches are useful for nematode diagnostics (Zijlstra *et al.*, 1997), direct comparison of ITS rDNA spacer sequences yield more detailed information about variation within and among nematode species (Pokharel *et al.*, 2005).

Traditional Methods

Morphology

Female

Elongate, slight terminal protuberance present. Stylet 12–15 mm long, basal knobs ovoid, offset. Perineal pattern rounded/ oval, striae smooth, lateral field absent.

Male

Labial region not offset, labial disc not elevated, lateral lips usually present. Stylet 15–20 mm long, basal knobs ovoid, offset. DGO = 3–4 mm.

J2

L = 410–480 mm, hemizonid anterior or adjacent to excretory pore, tail = 60–80 mm, tail tip finely rounded. (Perry *et al.*, 2010)

Dorso-ventral, oval to almost circular in shape, moderate in height of arch, no lateral incisures or gaps, tail tip marked with prominent, coarse, fairly well separated striae that sometimes form an irregular tail whorl (Pokharel *et al.*, 2007).

Differential host/Host range study

Variations in host range are known to occur in some species of root-knot nematodes, and attempts have been made to characterize these on the basis of differential host range. Sasser

(1954) proposed a simple method, based on responses to a series of differential hosts and the amount of galling induced, to identify four of the five species of *Meloidogyne* recognized by Chitwood (1949).

Subsequently, this test was often a component in descriptions of new species, purporting to demonstrate a 'unique' host reaction (Sasser, 1979). This test became known as the 'North Carolina differential host test' and has been used to detect host races within the 'Chitwood species' (Sasser and Carter, 1982).

The 'International *Meloidogyne* project' (IMP) summarized the responses of about 1000 populations of the four most common species of *Meloidogyne* and their races to differential hosts, and more details were provided by Taylor and Sasser (1978), Eisenback *et al.,* (1981).

The following hosts are used for differential host study; Barnyard grass (*Echinocloa crusgali* (L.) Beauv., a common weed in rice fields); jute cultivars Tosa and Deshi; cabbage (*Brassica oleracea* var. *capitata* DC.) cv. Dawirth Green; wheat (*Triticum aestivum* L.; different varieties); tomato (*Lycopersicon esculentum* Mill.) cultivars Rutgers, Money Maker and Cherry Large Red; oat (*Avena sativa* L.); barley (*Hordeum vulgare* L.); corn (*Zea mays* L.); and rye (*Secale cereale* L.). These plants were also inoculated with a known isolate of *M. graminis* for comparison.

The North Carolina host range test [tobacco (*Nicotiana tabacum* L.) cv. NC 95, watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai.) cv. Charlestone Grey, cotton (*Gossypium hirsutum* L.) cv. Deltapine 61, pepper (*Capsicum annuum* L.) cv. California Wonder, tomato cv. Rutgers and peanut (*Arachis hypogaea* L.) cv. Florunner) (Sasser and Triantaphyllou, 1977).

Biochemical methods

Isozymes

With increasing number of species under genus *Meloidogyne*, species identification is becoming a difficult task on the basis of morphological observations alone. Root knot nematode in second stage (J_2) is available abundantly from soil but identification of the species based on the morphology of J_2 cannot be made with certainty. Therefore, adult females are dissected out from galled roots for perineal pattern morphology. The males can be easily recovered from root and soil but are of uncertain population. Identification from J_2 can be achieved through regeneration of progeny on a susceptible host plant to attain full grown female. Further, to overcome the limitations of morphological characterization and differential host test, Nematologists are in search for alternative or supplementary tools for identification of *Meloidogyne* species. Currently, the number of species under the genus has inflated to such a number that comparison of all at a time is rendered difficult to distinguish them. Under such compelling situation, taxonomists are relying on novel approaches of taxonomy for this genus. The initial efforts were concentrated on biochemical and serological methods (Hussey, 1979). Nowadays, the species identification process involves morphological features and supplementary information on cytological, biochemical, physiological data (Hirschman, 1985), molecular data, host response and mechanisms of parasitism (Williamson and Hussey 1996). Dickson *et al.,* (1970) found stability in protein profile in root knot nematode and indicated its utilities in the identification of species. Later, the usefulness of disc-electrophoresis in species characterization was realised. Protein and enzyme composition patterns visualized through gel electrophoresis methods were found useful to identify *Meloidogyne* species

(Hussey, 1979; Esbenshade and Triantaphyllou, 1990). Among the four commonly studied enzyme patterns (non-specific esterase, malate dehydrogenase - Mdh, superoxide dismutase and glutamate-oxaloacetate transaminase), beta-esterase is considered to be the most useful for differentiating major *Meloidogyne* species (Cofcewicz *et al.,* 2004). One of the earliest examples of the use of isozyme phenotypes to distinguish *Meloidogyne* spp. was published by Esbenshade and Triantaphyllou (1990), who reported esterase patterns from 16

Meloidogyne species, with the most common phenotypes being A2 and A3 (*M. arenaria*), H1 (*M. hapla*), J_1 (*M. incognita*) and J_3 (*M. javanica*). Esterase phenotype is considered to be a useful taxonomic character, but requires adult females at a specific developmental stage for accurate diagnosis (Eshenshade and Trintaphyllou, 1990).

Swain and Prasad (1991) observed many bio-chemical changes in resistant and susceptible rice varieties to *M. graminicola.*

The amino acids alanine, aspartic acid, serine and methionine were more in resistant varieties (Udaya and Annada) compared to the susceptible variety (Parijat).

Molecular methods

There is no denying that PCR-based methodologies are of ever-increasing importance in species diagnostics and phylogeny within the genus *Meloidogyne*. Techniques include RFLP (restriction fragment length polymorphism) profiles of the ITS (internal transcribed spacer) region of rDNA, RAPD (random amplified polymorphic DNA) fragments, 18S rDNA sequences, satellite DNA probes and species-specific primers.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).

Gene sequencing and phylogenetic analyses

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).

Table.1 1 Day-second stage juvenile, 4 Day-third stage juvenile, 6 Day-fourth stage juvenile, 20 Day-adult female, 20 Day-eggs

Second Stage Juvenile	Longer (l=415-484µm), thicker (a=22-27), tail less pointed Rectum not dilated
Female	Larger (445-765 µm long & 275-520 µm dia). EPST=2.6 spheroid. Perineal pattern with whorl of striae around tail tip
Male	Longer (1==1000.2000 µm), lateral field with 6-8 lateral incisures.

Table.2 Root-Knot Index 0 to 5 scales for *Meloidogyne* spp. (Taylor and Sasser, 1978)

Grade	Description	Reaction
0	No galls	Immune
1	1-2 galls / root system	Resistant
2	3-10 galls / root system	Moderately resistant
3	11- 30 galls / root system	Moderately susceptible
4	31-100 galls / root system	Susceptible
5	>100 galls / root system	Highly susceptible

Plate.1 Nursery and main field showing uneven yellowish patches and galls on root system

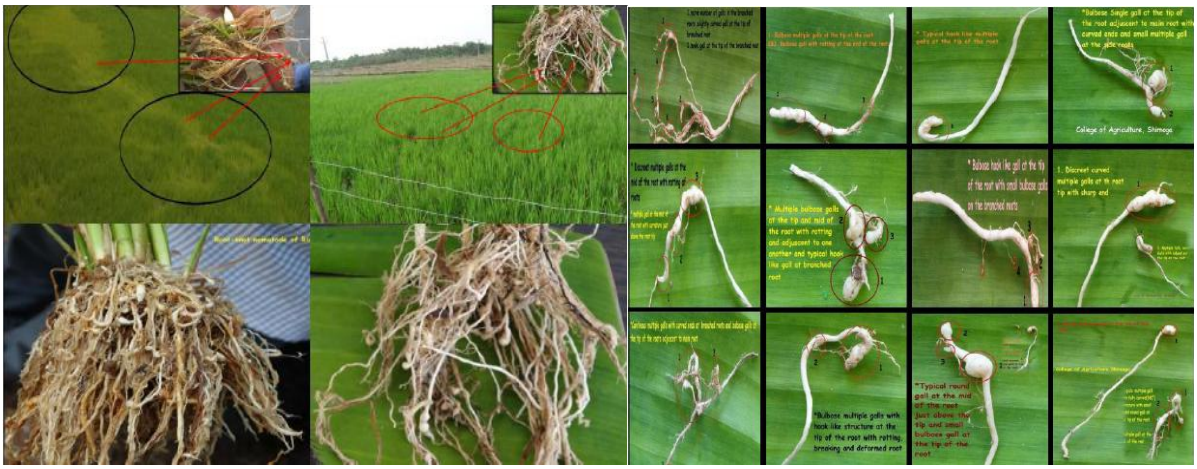

Plate.2 Different life stages of *Meloidogyne graminicola* at different days

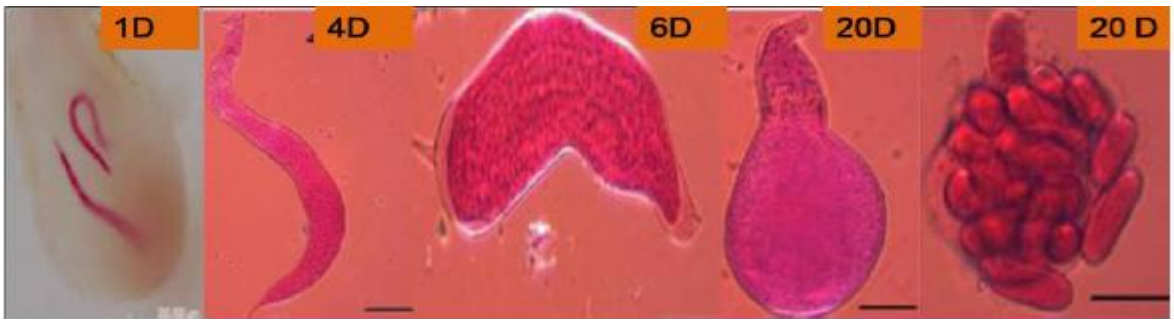

Plate.3 Galls induced by *M. graminicola* in rice roots. A, b and C: Historical analyses where root cross section (10m) at 4 (A, B) and 31 (C) days after infection were stained with toluidine blue. (D) Galled roots (Source: Dutta *et al.*, 2012)

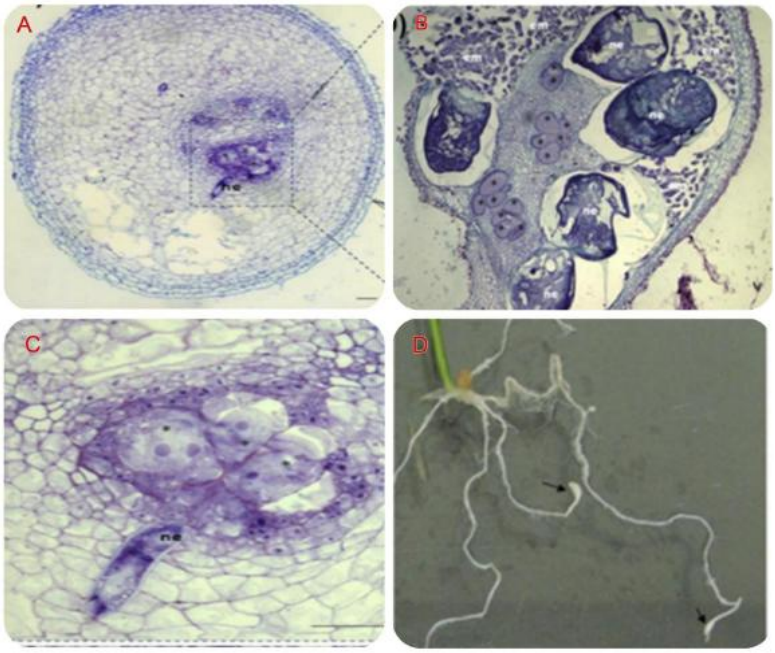

Plate.4 *Meloidogyne graminicola*. LM of male and second stage juvenile (J2). A: Entire male; B: Anterior region of male; C: Head end of male; D: Pharyngeal gland of male; E: Tail region of male; F: Entire J2; G: Anterior region of J2; H: Genital primordium; I, J: Tail regions of J2. (Scale bars: C-E, G-J = 10 µm; B = 20 µm; A = 150 µm) (Source: Text book; Root-knot nematodes by Perry *et al.*, 2010)

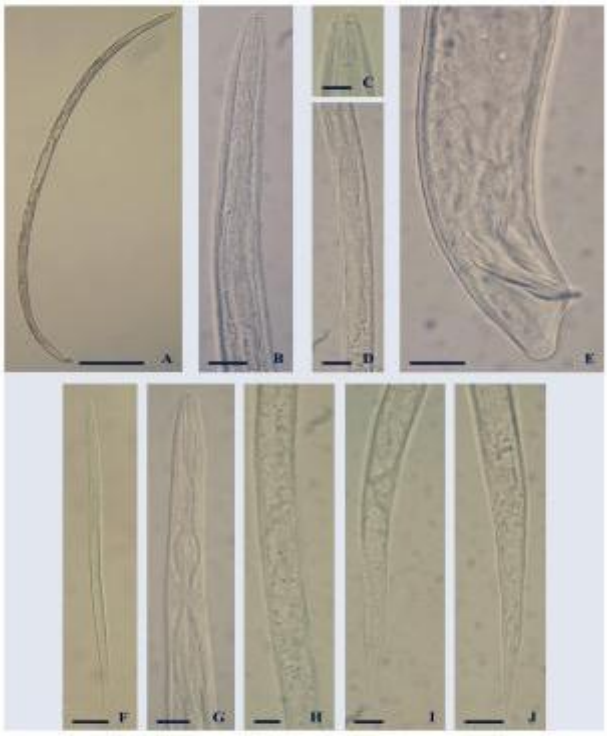

Plate.5 Perineal patterns (Source: Text book; Root-knot nematodes by Perry *et al.*, 2010)

As ITS sequence data for *M. graminicola* was not available in GenBank, two known *M. graminicola* isolates were also included in the study. One was from Bangladesh (*M. graminicola* BP3) (Padgham, and Ithaca, 2003), and the other (*M. graminicola* US) was from infected nut-sedge in Florida (provided by Dr. Janete A. Brito, Florida Department of Agriculture, Gainesville, FL). Sequence data for *M. graminis* was also unavailable in the GenBank, thus, a known *M. graminis* isolate obtained from turf grass near Buffalo, NY (provided by Dr. Nathaniel Mitkowski, University of Rhode Island), was also included in this study. Primers rDNA2 (5′-TTGATTACGTCCCTGCCCTTT-3′) and rDNA1.58s (5′-ACGAGCCCGAGTGATCC ACCG-3′) (Vrain *et al.*, 1992, Powers, 2004) obtained from Sigma Genosys Inc. (St. Louis, MO) were used in this investigation. The 33 isolates described above were utilized for this portion of the study. Eggs were obtained by the sodium hypochlorite method, allowed to hatch for 3 d at room temperature, and 60 J_2 were picked. The J_2 were then rinsed three times with sterile distilled water, crushed in 60 µl sterile distilled water on a sterile glass slide and transferred to a microfuge tube to be used as DNA template. Each DNA template was distributed into five tubes containing 12 µl of template/tube and frozen until PCR was performed. Each reaction was performed as previously described (Mitkowski *et al.*, 2003) and contained 16.25 µl water, 1.5 µl $MgCl_2$ (25 mM), 2.5 µl magnesium-free buffer (Promega, Madison, WI), 0.75 µl dNTP mix (200 µM each dA, dC, dG, dT) and 1.5 µl each primer (10 mM). Twelve microliters of DNA template was then added, and a drop of light mineral oil was placed in each tube to cover the reaction mixture. The reaction tubes were placed into an MJ PTC-100 thermal cycler (Waltham, MA) at 94°C, and 0.2 unit of Taq DNA polymerase (Promega) was added to each tube through mineral oil. The PCR cycle consisted of an initial step at 94°C for 2 min followed by 25 cycles of 94°C for 1 min, 47°C for 1 min and 72°C for 1 min and a final extension for 5 min at 72°C. The PCR products were purified with the Promega DNA cleaning kit and sequenced in both directions at the Cornell University Biotechnology Resource Center. The ITS sequences of the other *Meloidogyne* species included in the phylogenetic analyses were obtained from the GenBank (*M. chitwoodi* Golden, O'Bannon, Santo and Finley, AY593889; *M. javanica* (Treub) Chitwood, 26892; *M. minor* Karrsen *et al.*, AY53899; *M. incognita* (Kafoid and White) Chitwood, AY438556; *M. arenaria* (Neal) Chitwood, AF 387092; *M. hapla* Chitwood, AF576722; *M. naasi* Franklin, AY59301; and *M. trifoliophila* (Bernard and Eisenback, AF077091). The computer program NTI 6.0 (Infomax, Inc., Bethesda, MD) was used for sequence alignment. For amplification products that could not be sequenced directly, PCR products were cloned using the TA cloning kit (Invitrogen Corporation, Carlsbad, CA) according to manufacturer's instructions. Up to four clones from each isolate were sequenced. Phylogenetic analyses, including maximum

parsimony and neighbor-joining, were performed using PAUP*4.0b10 (Swofford, 2002) based on the partial ITS sequence alignment. Maximum parsimony analyses were performed via heuristic search options in PAUP*4.0b10 using the branch swapping algorithm (tree-bisection-reconnection). Neighbor-joining analysis was calculated with uncorrected "p" distance. Based on previous studies, *M. incognita* and *M. arenaria* were chosen as outgroup taxa (Castillo *et al.,* 2003). Bootstrap analyses of 1,000 replicates were conducted to assess the degree of support for each node on the tree. Pokharel *et al.,* (2007) undertook phylogenetic analysis based on the sequences of partial internal transcribed spacer (ITS) of the rRNA genes and indicated that all Nepalese isolates formed a distinct clade within known isolates of *M. graminicola.* The former study provides RNA–seq data obtained from roots infected with root rot nematode *H.oryzae* in comparison with uninfected roots and root galls induced by *M. graminicola*, at three and seven days after inoculation compared with control root tips (Kyndt *et al.*, 2012). Ji *et al.*, (2013) focus on the gene expression inside laser capture microdisected giant cells at 7 and 14 DAI and compared this profile with vascular cells from uninfected roots. In rice, *M. graminicola* able to suppress transcription of key immune regulatory genes (Kyndt *et al.*, 2012). *M. graminicola*effector proteins expressed in oesophageal gland cells are currently being analysed for their role in RKN- rice interaction (Nguyen, 2014) (Plate. 3).

Management

Host plant resistance

Based on the number of endoparasites and root-knots, the varieties Tkm 6, Patnai 6, and N 136 were graded as resistant (Israel and Rao 1971). Roy (1975) graded the varieties Garem and Dumai as resistant in Assam since knotted roots were absent and 11 others including Jagannath and IR 20 were rated as tolerant as they developed up to 5 per cent galls. Yik and Birchfield (1979) observed that *M. graminicola* readily reproduced on 21 cultivars out of 26 cultivars. Based on root gall development, two cultivars showed resistance to the rice root-knot nematode and nine cultivars were mildly resistant, and 15 were susceptible. Arayarungsarit *et al.,* (1985) reported that three selections from IR36/RD25 crosses showed resistance to *Meloidogyne* spp. in Thailand. Swain *et al.,* (1986) reported that Daya, Udaya, MW 10 and IR 36 were found to be resistant to *M. graminicola*. Bose *et al.,* (1998) conducted RAPD analysis on five rice cultivars including three highly resistant (Ramakrishna, Rasi and Kalarata) and two highly susceptible (Annapurna and Kiran) to *M. graminicola*. The highest polymorphism was recorded between Annapurna and Ramakrishna. They suggested that cross combination of Annapurna and Ramakrishna could prove useful for mapping the *M. graminicola* resistance gene in rice. Debanand (1999) screened 25 rice cultivars against *M. graminicola,* under field conditions in India, out of which only MTC 23/A showed resistance. Soriano *et al.,* (1999) observed that one accession of *O. longistaminata* A. Chev. represented by two individuals (WL02-2 and WL02-15) and threeaccessions of *O. glaberrima* (TOG7235, TOG5674 and TOG5675) were resistant to *M. graminicola* whereas, all the *O. sativa* accessions were susceptible. Hassan *et al.,* (2004) reported that rice varieties Loknath 505 and M-36 were found to be highly resistant to the rice root-knot nematode, *M. graminicola* from Allahabad. Poudyal *et al.,* (2004) reported that all the cultivars tested were susceptible *to M. graminicola* except Masuli and Chaite-6, which were moderately resistant. Anil Prashar *et al.,* (2004) clearly demonstrated that the

severity of *Meloidogyne graminicola* to rice increases with increase of water stress, hence the important of using rice cultivars that are tolerant to water stress and resistant to the nematode. Kalita *et al.,* (2004) screened twelve commonly cultivated rice cultivars against rice root knot nematode (*Meloidogyne graminicola*) in sick soil under greenhouse condition. Gitanjali and Thakur (2007) reported that from a greenhouse experiment conducted to evaluate the resistance of rice germplasm /cultivars Lampnah, 1, RCPL 1-87-8, RCM 9, IR 36, TOX 3093-10-2-3, JTA 222, Manipuri local and Shahsarang to root-knot nematode (*Meloidogyne graminicola*). Manipuri local, TOX-3093-10-2-3 and Lampnah-1 were recorded as moderately resistant. RCM-9, IR-36, JT A-222 were recorded as susceptible, whereas, Shahsarang and RCPL-1-87-8 were highly susceptible. Simon (2009) evaluated the susceptibility of 53 rice genotypes to *M. graminicola* in field and pot experiments and observed that 13 cultivars were highly resistant to this nematode. Ajai Srivastava *et al.,* (2011) screened eighty seven cultivars of rice and fifty nine cultivars of wheat against root-knot nematode infection in the field during *kharif* 2007, 2008 and 2009 and *rabi*2008 and 2009. The study revealed that two rice cultivars Achhoo and Naggardhan and two wheat cultivars HS 295 and VL 829 as resistant with 2 score. Rice lines Ranbir Basmati, Hasan Sarai, and Purple cultures and wheat cultivar HS 240 were rated as susceptible. KMP- 179 was found to be resisitant (Ravindra, 2012) and in addition to this, out of 145 local cultivars, 32 and 45 local cultivars were found to be highly resistant and resistant respectively against rice root knot nematodes (Ravindra *et al.,* 2015). Narasimhamurthy (2014) screened twenty rice genotypes against *M.graminicola* Out of twenty genotypes, only one genotype KMP-179 was found to be highly resistant which recorded least root-knot index (1.6), while, 4 genotypes viz., MAS-26, BR-2665, MTU-1001 and KMP-194 were moderately resistant with root-knot indices between 2 and 3. However, 11 genotypes viz., KMP-175, MAS-946, KMP-149, IR-64, MTU1010, JGL-1798, KCP-1, KMP-153, Kadamba, KMP-169 and Rasi which were found to be susceptible with root-knot indices varying between 3 and 4. The remaining 4 genotypes, KMP-148, KMP-128, KMP-105 and Thanu were learnt to be highly susceptible. Joymati Devi (2014), screened ten varieties, which showed evidence of damaging potential of *Meloidogyne graminicola* in terms of plant growth parameters and disease incidence.

Disease intensity grade was classified on the basis of root knot index. All the varieties were susceptible to *Meloidogyne graminicola* except Dharam and Tampha which were resistant. Maximum numbers of root gall (45) were recorded in rice variety Lamyanba whereas minimum root galls (2) were recorded in rice variety Dharam.

Biological control

Pathak and Kumar (1995) Showed maximum mortality (>96%) of *M. graminicola* juveniles when exposed to culture filtrates (100 and 50% conc.) of *Trichoderma harzianum* Rifai. Poudyal *et al.,* (2001) reported that the application of organic amendments revealed there was no significant difference in plant height, tiller number, fresh root weight and root length. But significantly reduces root knot index, second stage juveniles in soil and roots and egg numbers in roots. Application of *Pseudomonas flourescens* at 20 g/m2 was found to be effective in reducing the nematode numbers and increasing the grain yields (Anon, 2003). Anitha and Rajendran (2005) reported that integration of *P. fluorescens* (2.5 kg/ha), neem cake (1 ton/ha) and carbofuran (1 kg a.i/ha) was highly effective in improving plant growth and yield

in the nursery as well as in main field. All the three treatments were compatible with each other and caused significant reduction in the population of *M. graminicola*. In *in-vivo* screening tests, *Bacillus megaterium* significantly reduced nematode galling and J2 penetration compared with uninoculated controls. Additionally, in *in-vitro* tests, using culture filtrates of *B. megaterium* significantly delayed nematode egg hatch and reduced J2 mobility (Padgham *et al.,* 2005).

Anita and Samiyappan (2012) reported that induction of defense enzymes phenol, peroxidase (PO), polyphenol oxidase (PPO), phenyl ammonia lyase (PAL), super oxide dismutase (SOD) and chitinase by *Pseudomonas fluorescens* isolate Pf1, on rice root-knot nematode resulting in significant reduction in nematode infection. Isolates of endophytic and rhizosphere fungi *viz.*, *Fusarium* and *Trichoderma* are the potential biological control agents against *M. graminicola* in rice (Le *et al.,* 2009). Seenivasan *et al.,* (2012) reported that the mixtures of *P. fluorescens* strains with PF1+TDK1+PY15 significantly reduced *M. graminicola* infestation when applied as bacterial suspensions through seed treatment.

Cultural control

Prot *et al.,* (1994) positively correlated the nitrogen concentration in roots with initial population and the number of juveniles of *M. graminicola* recovered from the roots. They observed that nitrogen application increased growth and yield whether plants were infested by the nematode or not.

However, since the percent of yield loss remained approximately constant for a given initial population across the range of nitrogen quantities applied, nitrogen applications do not reduce the relative nematode effect. Soil amendments with decaffeinated tea waste or water hyacinth compost (300 or 600g/4.5 kg soil) reduced root-knot nematode infestation and increased plant growth (Roy, 1976). Rice-mustard-rice crop sequence, followed by rice-maize-rice and rice-fallow-rice were effective in reducing nematode development (Kalita and Phukan, 1996). A drastic decline of 98 and 94% in the population of *Meloidogyne* spp. and *Hirshmanniella oryzae* respectively, were recorded when the rice crop was rotated with brinjal (Ramakrishnan, 1995). Crop rotation with non-host crops *viz.*, sweet potato, cowpea, sesamum, castor, sunflower, soybean, turnip and cauliflower inhibit nematode development (Rao *et al.,* 1984; Rao, 1985). Polthanee and Yamazaki (1996) observed that *in situ* green manuring with marigold suppresses root galling and increases rice grain yield by 46% over the untreated check.

The increase in yield was attributed to a reduction of nematode densities in soil by marigold. In addition, marigold plant materials may serve as organic manure and provide nutrients for rice growth. Burning of 15 cm deep rice hulls significantly reduce *M. graminicola* populations in the soil (Gergon *et al.,* 2001). Compared with continuous rice treatments (averaged over burning and mulching treatments), treatments with fallow or cowpeas in the previous year had 32% less herbaceous weed biomass, 90% fewer *A. conyzoides* and over 99% fewer *M. graminicola* in field trials (Roder *et al.,* 1998). In Philippines, rice based cropping sequences such as rice-mungbean, corn-cabbage rice, rice-tobacco-rice, rice-watermelon-rice, rice-cotton-rice, have been found effective in combating root-knot nematode menace in rice (Davide and Zorilla, 1983). The root-knot problem in paddy can be managed by growing healthy and disinfested nursery, following summer ploughing and puddling of main fields before transplanting. Crop rotation with non-host crops like jute,

mustard, chickpea and resistant varieties reduces *M. graminicola* infestation (Pankaj *et al.,* 2010)

Chemical control

Krishnaprasad and Rao (1976) reported that a soil drench to infected crops with oxamyl, fensulfothion or carbofuran at 1Kg a.i. /ha resulted in complete control of both the soil and root population of *M. graminicola.* Carbofuranbelow 250 ppm had persistent toxicity and egg mass production was inhibited (Krishnaprasad and Rao, 1980). Rahman and Taylor, 1983 reported that, *M. graminicola* infestation was reduced by the application of carbofuran at 3Kg a.i. /ha incorporated at sowing in deepwater rice. Krishnaprasad and Rao (1984) indicated that Oxamyl at 500 to 1000 ppm when applied as foliar sprays, were effective in reducing *M. graminicola* followed by soil application of phorate and carbofuran at 1 kg a.i./ha. Anon (1985) application of carbofuran to soil in nursery and main field at the rate of 1Kg a.i. /ha reduced *M. graminicola* by over 90 per cent and resulted in increased yield of about 100 per cent.

Lopez and Salazar (1989) found fenamiphos (at 6 kg a.i. /ha) to reduce root-knot index of *M. salasi* in rice (Table 2). Panigrahi and Mishra (1995) revealed that carbofuran, phorate, isazophos, cartap and carbosulfan or quinalphos when given as soil application at 1 kg a.i. /ha significantly reduces the root galling by*M. graminicola.* Imelda and Georges (2003) reported that the assessment of crop rotation, fallow and nematicide treatments in naturally infested fields to manage *M. graminicola* populations and prevent yield losses. Carbofuran improved yield of the first rice crop but did not affect the second rice crop.

Application of Phosphonothioate 10G at 1 kg a.i./ha at 7 days prior to uprooting plus main field application at 45 days after transplanting at 1 kg a.i./ha exhibited maximum reduction both *M. graminicola* and produces maximum yield (Das and Choudhury, 2012).

Transgenic nematode resistant plants

Transgenic resistances against a wide range of nematodes have been developed, based on an antifeedant, approach that uses plant cystatins. Cystatins are naturally occurring proteinase inhibitors which are involved in plant defences against insects and pests. Furthermore cystatins, are not involved with mammalian digestive enzymes (Atkinson *et al.,* 1995). Cystatins are also found in a wide range of foods such as those found in rice seeds which are eaten daily by thousands of people, other sources include maize and sunflower. Cystatins are not normally produced in roots where the nematodes attack but plants can be modified by the use of root specific promoters driving the expression of cystatin in areas of the plants where the nematode infest. This has the added advantage of ensuring the cystatin is not expressed in the edible plant parts or green tissue. Transgenic expression in plant rots reduces nematode growth, development and fecundity. The anti-nematode defence offers major practical benefits for use in the developing world. It offers the ability to control several nematodes attacking a crop such as rice concurrently or at different locations without any need for awareness of nematode problem at grower level. This is useful, as rice roots tend not to gall upon nematode infestations. Using a genotype independent biolystic transformation system twenty five transformed clones containing an engineered cysteine proteinase inhibitor oryzacystatin-IDD86, hygromycin resistant (aphIV) and β–glucuoronidase (gusA) gene were recovered from four varieties of rice. Up to 0.2% total soluble protein was detected by

western analysis.

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).

Integrated Disease management

Soil solarisation of nursery beds for 15 days and application of carbofuran 3Gat 15g/m2 to the nursery for effective management of root-knot nematode (Ravindra, 2006). Khan*et al.,* (2012) reported that root-dip and soil application of phorate 10G (25 mg a.i. /pot), carbofuran 3G (83.3mg a.i./pot), carbosulfan 20EC (5µL/pot) and chlorpyriphos 20 EC (6.25 µL/pot) reduces root-knot infestation in rice.

Somasekhara *et al.,* (2012) showed that the adoption of INMT (Integrated Nematode Management Technology) resulted in reducing the nematode population from 320 J2/200 cc soil as initial nematode population to 135 (cabrofuran (0.3 g a.i/m2) (T1), 165 (*Pseudomonas fluorescence* at 20 g/m2 (T2) and 192 (*Trichoderma viride* at 4 g (T3)/200 cc of soil and also increases yield 4.72 tonnes/ha, 4.67 tonnes/ha and 4.29 tonnes /ha in T1, T2 and T3 respectively, in comparison to 3.81 tonnes/ha in untreated control (T4). Ziaul (2013), reported that the soil application and root dip of *P. fluorescens* or *T. harzianum* + carbofuran was found most effective and suppressed the gall formation (40-46%), egg mass production (45-57%) and soil population (56-64%) of *M. graminicola,* and increased the plant growth variables by 37-42%. The combined treatment of carbofuran + *P. fluorescens* as root dip and single soil application (15 DAP) of carbosulfan or carbofuran was found highly effective against the nematode and suppressed root-knot severity by 65-72% and increased the yield by 30-35% in yield compared to epidemic condition. Integrated nematode management study was conducted in Assam, the pooled data of five years showed that carbofuran treated nursery bed had 100 galls/20 seedlings, *P. fluorescens* and *T. viridae* had 115 galls/20 seedlings and 118 galls/20 seedling. 29.6% and 28.6% yield increased in carbofuran and *P. fluorescens* treated plots (Sehgal *et al.,* 2014).Treatment combination of *Pseudomonas fluorescens* at 20g/m² + cabrofuran (0.3 g a.i/m2) recorded highest plant height (83.26 cm), root length (20.60 cm), maximum grain yield (44.1 q/ha) and least nematode population (132.67/200g soil) with reduction of 79.34% nematode population followed by *T. viride* at 20g/m²+ carbofuran with plant height (81.67cm), root length (18.50 cm), grain yield (43.6 q/ha) with least nematode population (198.00/200g soil) with reduction of 69.17% nematode population (Narasimhamurthy *et al.,* 2016)

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