Introduction

Rice root-knot nematode (*Meloidogyne graminicola* Birchfield, 1965) is the most important nematode in rice production and also other crops. The severity and importance of this nematode have increased in recent years in SE Asia, constraining rice-wheat production. Recently, research efforts of the Soil Management, Collaborating Research Support Project (CRSP) at Cornell University (Dr. John Duxbury, PI) in Nepal and other countries in South Asia identified the root-knot nematode as an important problem in rice based production systems (Duxbury, 2002).

Rice is a staple food crop that accounts for more than 50% of the caloric intake of people, 20% of the agricultural gross domestic production (GDP), and 34% of the total grain production in Nepal. Wheat is the third ranked cereal crop in the country and is mostly grown in lowland areas after rice; about 80 to 85% of the wheat grown in Nepal is grown as a rice-wheat rotation. The average productivity of rice in Nepal is 1.9 t per ha, which is below the average productivity of neighboring countries including China (3.5 t per ha) and India and Pakistan (2.4 t per ha). Average yield of wheat in Nepal is 1.6 t/ha, which is also below the average yield of other South Asian countries (Kataki et al., 2001). Several factors are responsible for the low productivity of rice and wheat. Of these, availability of irrigation water, soil nutrient status and outbreaks of insect pests and diseases are major constraints to higher productivity (Pimentel, 1983). However, Kataki et al. (2001) reported that pests and diseases to be the number one problem (ranked 1st) in rice out of 4 major problems identified in a survey of farmers, whereas pests and diseases were ranked 9th out of 10 problems identified in wheat production. Despite an increasing trend in the use of inputs and the adoption of new technologies, productivity of rice and wheat is not responding in terms of yield growth, as they should be. The per unit productivity growth is declining in some
locations and yield is less than that found in other countries in the region with the result that food deficits occur in most years. Blast, bacterial blight, and sheath blight are among the important diseases of rice; whereas rust, leaf blight and loose smut are prevalent on wheat (Dahal et al., 1992). Nematodes that feed on roots and generally do not produce specific above-ground symptoms are also possible causal candidates for this decline. However, they are often neglected due to lack of conspicuous above-ground symptoms and knowledge of plant-parasitic nematodes.

More than 200 species of plant-parasitic nematodes (PPN) have been reported to be associated with rice worldwide (Prot, 1994). Among these nematodes, root-knot nematode (*Meloidogyne* sp.) is considered as the major problem in rainfed, upland and lowland rice production regions, whereas rice root nematode (*Hirschmanniella* sp.) is a problem on lowland rice only in South and Southeast Asia (Prot et al., 1994). The root-knot nematode (primarily, *Meloidogyne graminicola*) has been reported to cause economic loss in upland, lowland and deep-water rice and also in rice nurseries (Bridge et al., 1990). However, prevalence and severity of other root-knot nematode species in rice and/or wheat could not be overlooked. *M. oryzae* in Surinam (Maa, 1978) and *M. tritici* in India (Gaur and Sharma, 1999) were known to attack rice and grasses resulting in similar symptoms as those caused by *M. graminicola*. In addition, other root-knot nematode species (*M. aranaria*, *M. incognita* and *M. javonica*) have been also reported attacking rice in upland production region (Jairajpuri and Baqri, 1992).

Species of *Pratylenchus*, *Heterodera*, *Helicotylenchus* and *Meloidogyne* are the major plant-parasitic nematodes on wheat in upland production areas, whereas the root-knot nematode (*Meloidogyne graminicola*) is considered as the major nematode in lowland rice-wheat rotation (Sharma et al., 2001). In addition, other root-knot nematodes (*M. artellia* and *M. nasii*) have also been reported attacking wheat
The root-knot nematode observed in rice-wheat production fields in Nepal were suspected to be *M. graminicola* based on symptom production and nematode survival in flooded soil condition. A literature search revealed that a root-knot nematode (*M. trifoliophila*) was similar to *M. graminicola* in morphology and perineal pattern, but *M. trifoliophila* did not infect cereals. Bernard and Eisenback (1997) recognized a group of *M. graminicola* associated with white clover, but not attacking rice, wheat and grasses as *M. trifoliophila*.

*M. graminicola* was reported for the first time on the grasses *Poa annua*, *Alopecurus carolinianus*, *Elusine indica* and *Echinocloa colonum*, and oat and also common beans (*Phaseolus vulgaris*). This nematode has been reported from USA in the states of Louisiana, Georgia, and recently in Florida on sandbur (*Cenchrus* spp.) (Brichfield, 1965; Minton et al., 1987; Hondoo, et al., 2003; Brito et al., 2004; Power et al., 2005). However, this nematode has been studied extensively on rice in several Asian countries. *M. graminicola* was reported to have more than 98 host plants that includes cultivated crops and weed species (McGowon et al., 1989). Recently, this nematode has been found to reduce onion yield by 16-35% in the Philippines (Gergon et al., 2002) and is considered a potential pathogen in wheat in Nepal, India, Pakistan and Bangladesh (Pokharel et al., 2004; Gaur and Sharma, 1999; Somorro and Hauge, 1992; Padgham et al., 2004). In addition, *M. graminicola* was found to infect many other crops like cowpea, lentil, black-gram and many weeds that grow after rice in the same field. Also, symptoms produced on rice and survival of the nematode were different in flooded and non-flooded soil conditions (Padgham, 2003; Pokharel, unpublished). Furthermore, detailed information on the identity and biology of root-knot nematode in rice production fields as well as practical management of the nematode were limited in Nepal. Thus, the interest to initiate this investigation on...
nematode isolates characterization and search for sources of resistance in rice and wheat.

In rice, PPN can cause up to 10% yield loss worldwide (Prot, 1994). However, root-knot nematode alone is capable of causing up to 50% yield loss in rice in many production regions (Lorenzana et al., 1998). It was reported that this nematode reduced rice yield by 30% and 16-20% in farmers fields in Nepal and Bangladesh, respectively (Duxbury, 2002; Padgham, 2003), and 31-97% growth reduction under greenhouse condition in Nepal (Sharma-Poudyal et al., 2005). Infection severity and yield loss caused by this nematode in rice seem to be influenced by several other factors including initial inoculum density. In addition, Soriano et al. (2000) reported that low nitrogen content of sandy soil might also increase the susceptibility of plants to nematode damage and they also concluded that the tolerance level of rice cultivars to *M. graminicola* vary with water management systems. Similarly, crop rotation with non-host crops to this nematode significantly affected the yield losses observed in rice.

Wheat was found to support a high population of this nematode in between two crops of rice, thus increasing the severity of this nematode in rice-wheat cropping systems (Padgham et al., 2004; Gaur and Sharma, 1999). Selected cropping sequences and weed management in a rice-based crop rotation seems crucial in the management of this nematode. Unfortunately, research focused on this nematode in the rice-wheat production system was negligible despite the fact that the root-knot nematode has become increasingly important in the productivity of rice-wheat systems in SE Asia (Gaur et al., 1993). Results of a preliminary pathogenicity test confirmed the damage of this nematode to both rice and wheat causing almost 40% reductions in root and shoot length of rice seedlings grown in *M. graminicola* infested soil (Pokharel, unpublished).
Molecular tools have been applied in the recent past to identify plant-parasitic nematodes including *Meloidogyne* species, but not *M. graminicola*. Moreover, information on the genetic variability within *M. graminicola* was lacking, but a limited information on morphometric variability and the biology of this nematode was available in the literature, but not in Nepal. Because of the ability of *M. graminicola* to reproduce both parthenogentically and sexually (Triantaphyllau, 1985), it is possible that the species is genetically diverse and adaptable to various environmental conditions. If a high degree of genetic diversity exists within *M. graminicola*, it is then possible to observe variability in pathogenicity and virulence of isolates of this nematode. This might explains why it has been difficult to develop alternative management strategies against this nematode, specially the identification and development of resistant germplasms. Accurate identification of nematodes is central to many fundamental and applied areas in nematology, but is frequently difficult using traditional diagnostic approaches. Biochemical and molecular methods have provided alternative taxonomic tools (Gasser, 2001). Such techniques generally have high discriminatory powers, are often inexpensive and can be user friendly, even for the non-specialist. Many molecular techniques have been shown to be valuable tools for the identification of root-knot nematode such as RAPD-PCR, restriction fragment length polymorphism (RFLPs) (Power, 2004), Amplified sequences of mitochondrial DNA (mtDNA, Powers and Harris, 1993), sequence differences of rDNA (Zijlstra et al., 1995) and ITS fragment (Zijlstra, 1997). In particular, the PCR method has revolutionized nematode taxonomy and genetics, mainly because its sensitivity permits the amplification of gene(s) fragments from minute amounts of genomic DNA. Mitkowski et al. (2003) reported variability in *M. hapla* populations from New York State by comparing the ITS region of individuals of each populations. ITS is simple and informative tool and universal ITS primers are available for PPN.
However, ITS may perform well as a diagnostic tool for *Meloidogyne* species that reproduce sexually or by facultative parthenogenesis. It is clear from the research of Hugall et al. (1999) that the use of the ITS region for identification of the mitotically parthenogenesis species could easily lead to mis-diagnosis. However, neither the ITS sequences of this species were available nor information on the usefulness of ITS sequences in the identification of this nematode were known.

Management of the root-knot nematode with chemicals is difficult not only due to health and environmental hazards associated with their use, but also to their high cost that make them economically prohibitive to low income farmers in South Asian countries. Thus, the use of resistant varieties is more sustainable and safe method of nematode control to these growers. A number of the rice varieties has been reported as resistant to isolates of *M. graminicola* from Louisiana, USA (Yik and Brichfield, 1979), India (Roy, 1973) and Indonesia (Bridge et al., 1990). However, other studies have failed to identify rice germplasm with resistance to this nematode (Taya and Dabour, 2004; Chunram, 1981; Rao et al., 1986, Prasad et al., 1986). Reaction of the vast majority of rice and/or wheat varieties developed by the International Rice Research Institute (IRRI), Center for Maize and wheat Development (CIMMYT) and the national research centers of Asian countries to the root-knot nematode are not known. Moreover, IRRI has developed information on the resistance of rice germplasm to various pests and diseases, but not to this nematode. Resistant sources to *M. graminicola* have been identified in wild-rice (Soriano et al., 1999), but are difficult to transfer into commercial rice varieties. Several attempts made previously to incorporate the resistance in wild rice (*O. glaberima*) into cultivated rice (*O. sativa*) germplasm were not encouraging. Although *O. glaberrima* was highly resistant to *M. graminicola*, the tested inter-specific progeny did not express the same level of resistance as the resistant parent, indicating a need for
further back-crossing to get acceptable resistant progenies (Plowright et al., 1999). Identification and use of more than one resistance source is often needed for the durability of the resistant germplasm. Thus, the search for resistant germplasm against this nematode among the cultivated rice landraces and varieties is needed. Also, the reaction of locally adopted wheat germplasms to this nematode is not known and very limited information is available on the resistance of wheat in general to this nematode. Rice varieties resistant to other pests and diseases may also have resistance to this nematode, thus warrant evaluation. Multiple resistance has been identified in many host plants against different groups of plant pathogens and/or pests. Fehr (1987) recommended the search for resistance in commercial cultivars first, then parents of hybrid cultivars or parent of synthetic cultivars, and finally the elite breeding lines that may soon become cultivars or acceptable breeding lines with superior one or more characters. If no resistance source is found, the search may then focus on wild relatives.

Wheat is grown in the same fields after rice and is highly susceptible to the root-knot nematode. However, the exact impact of this nematode on yield potential of wheat is still not clear. Also, the level of susceptibility of wheat varieties to *M. graminicola* is not well known. However, 4 and 10 wheat varieties from Bangladesh and Nepal, respectively were reported to be highly susceptible to an isolate of *M. graminicola* from Bangladesh (Padgham, 2003; Pokharel et al., 2004a); whereas 19 wheat varieties from India were considered as moderately resistant to an Indian isolate of this nematode (Taya and Dabour, 2004). Growing moderately resistance rice and/or wheat varieties in the same field over different seasons will likely reduce the nematode populations compared to growing highly susceptible rice and wheat varieties. The above suggested the need for an investigation to ascertain the identity of the species of root-knot nematode observed in rice-wheat production fields in Nepal and to assess
the reaction of rice and wheat varieties to this nematode. The present study was initiated with the following objectives.

1. Morphological, pathological, and molecular characterization of the population of root-knot nematode(s) collected from rice-wheat production fields in Nepal. Also, to compare the Nepalese isolates of root-knot nematode to similar isolates from other production areas (Bangladesh, Nepal, India and US),

2. To develop an effective screening protocol for assessing the reaction of rice and wheat germplasm to *M. graminicola*, and

3. To determine the reaction of selected germplasm of rice and wheat for resistance to the characterized isolates of root-knot nematode in Nepal. Also, to investigate the possible existence of an isolate by germplasm interaction among the tested isolates of *M. graminicola* and rice and wheat varieties.
Literature cited


CHAPTER 1

CHARACTERIZATION OF ROOT-KNOT NEMATODES FROM RICE-WHEAT PRODUCTION FIELDS IN NEPAL AND VARIABILITY OF MELOIDOGYNE GRAMINICOLA ISOLATES FROM DIFFERENT GEOGRAPHIC AREAS

Abstract

Thirty-three isolates of root-knot nematode were recovered from soil samples collected from rice-wheat fields in Nepal. Eggs from a gall, the progeny of a single female were obtained and inoculated onto rice cv. BR 11. These isolates were characterized morphologically, pathologically and molecularly in order to determine the identity of each isolate. The results obtained indicated a general phenotypic similarity (larval measurements, perennial pattern, host range and galls shape) of the Nepalese root-knot isolates with that of Meloidogyne graminicola. However, minor variations in larval measurements were observed among individuals of a number of the Nepalese isolates that were beyond the reported ranges of larval measurements for M. graminicola. Although the perineal patterns of the Nepalese isolates were similar to that of M. graminicola, there were four minor variations identified. The host range test conducted confirmed that the Nepalese root-knot isolates were not M. aranaria, M. incognita, M. javonica, M. oryzae or M. trifoliiophila. Both the rice varieties (LA 110 and Labelle) used to differentiate the virulence and aggressiveness of the Nepalese isolates were susceptible to all of the isolates. However, variability in the aggressiveness of the Nepalese isolates was observed. The internal transcribed spacer (ITS) sequences of the Nepalese isolates were unique and distinctly different from

*This chapter will be submitted to Journal of Nematology for publication.
other species of *Meloidogyne* and even from the closely related species, *M. graminis*. These sequences formed a single clade, along with Florida and Bangladesh isolates of *M. graminicola*. All Nepalese isolates within the clade formed two distinct haplotypes, one including the isolates from the hill region and the other with isolates from the plain region. Variability of isolates of *M. graminicola* obtained one from Florida (USA), and three each from Bangladesh, Nepal and India was compared by morphometric measurements of 40 randomly picked second stage juveniles and perineal patterns from 10 randomly picked mature females per isolate. In addition, host range and aggressiveness tests as well as the amplification and sequencing of internally transcribed spacer regions were also compared. The results exhibited minor variations on morphometric measurements and in perineal patterns among the isolates, but a significant variation in pathogenic ability and in host range. All isolates of *M. graminicola* except the Florida isolate were pathogenic to all the rice germplasms tested whereas all the isolates were pathogenic to the wheat varieties tested. These results suggested the existence of different races or pathotypes of *M. graminicola*. However, these isolates formed single clade in the phylogenetic analysis of internally transcribed spacer region (ITS) with some minor sequence variability.

**Introduction**

The average yields of rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) in Nepal are below the average yields obtained in neighboring countries including China, India and Pakistan (Kataki et al., 2001). Several factors are responsible for the low productivity of rice and wheat in Nepal. Of these, availability of irrigation water, soil nutrient status and outbreaks of insect pests and diseases are major constraints to higher productivity (Pimental, 1983; Kataki et al., 2001). Despite the increasing trend
in the use of production inputs and the adoption of new technologies, productivity of rice and wheat is not responding in terms of yield growth, as they should be. The per unit productivity growth is declining in some locations and yield is less than that found in other developed countries in the region, often resulting in food deficits. Blast, bacterial blight, and sheath blight are among the important diseases of rice, while rust, leaf blight and loose smut are prevalent on wheat (Dahal et al., 1992). Plant-parasitic nematodes are also possible causal candidates contributing to the observed yield decline. However, they are often neglected due to lack of conspicuous above ground symptoms.

More than 200 species of plant-parasitic nematodes have been reported to be associated with rice worldwide (Prot, 1994). Among these nematodes, root-knot nematode (*Meloidogyne* spp.) is considered to be the major problem in rainfed upland and lowland rice producing regions, whereas rice root nematode (*Hirschmanniella* spp.) is a problem on lowland rice only in South and Southeast Asia (Prot et al., 1994). Though several *Meloidogyne* spp. are known to attack cereals worldwide, *M. graminicola* is the only species reported to be adapted to flooded rice soils and is common in countries in South East Asia, including Nepal. This nematode (*M. graminicola*) was reported to cause up to 50% yield loss in rice in the Philippines (Lorenzana et al., 1998). In Nepal, limited information is available on the economic damage caused by plant-parasitic nematodes, especially on rice.

The species of root-knot nematode found on rice have been generally reported to be *M. graminicola*, based largely on the symptoms (hook-like galls produced on rice roots) and the ability of this species to adapt to lowland rice condition. Although other *Meloidogyne* spp. are known to infect rice, they are not known to survive under lowland production conditions and also are not known to produce the hook-like galls. Although the root-knot nematodes attacking rice in Nepal have been reported as *M.
*graminicola*, an accurate identification and information on their variability is still lacking. Accurate identification of nematodes is important and is central to understanding the host-parasite relationships, nematode damage and to implement appropriate management options. Traditional methods of nematode identification for root-knot nematode are based on morphology (Jepson, 1985), cytogenetics (Triantaphyllou, 1985), and a differential host range test (Taylor and Sasser, 1978). However these methods are not completely reliable and are time consuming (Stanton et al., 1997). Esterase phenotype is considered to be a useful taxonomic character (Esbenshade and Triantaphyllou, 1990), but requires adult females at a specific developmental stage for accurate diagnosis. DNA sequences of internal transcribed spacer (ITS) regions have been used successfully to diagnose species and populations of nematodes (Bloc et al., 1997; Cherry et al., 1997; Ferris et al., 1998; Ibrahim et al., 1997; Szalanski et al., 1997). These spacer sequences yield more detailed information about variation within and among nematode species than PCR- RFLP approaches (Nguyen et al., 2001).

This nematode was also reported on grasses in the states of Louisiana, Texas, Georgia and Florida of USA (Brachifield, 1965; Minton et al., 1987; Hondoo et al., 2003; Brito et al., 2004; Power et al., 2005), but extensively on rice grown in Asian countries (Bridge et al., 1990). However, the severity of the problem on rice in SE Asia was not well known until the research of the Soil Management CRISP project demonstrated that the root-knot nematode is causing > 30% yield losses in rice in farmer’s fields in Nepal and Bangladesh (Duxbury, 2002). In addition, this nematode was reported occurring on several crop and weed species (Mulk, 1976; MacGowan and Langden, 1989). Gergon et al. (2003) reported that *M. graminicola* is a problem in onion production in the Philippines and is reducing yield between 9 to 36%. Recently, it was also considered a potential pathogen on wheat in Pakistan, India, Bangladesh
and Nepal (Soomro and Hague, 1992; Gaur and Sharma, 1999, Padgham et al., 2004; Pokharel et al., 2005), especially in rice-wheat production systems.

This project was conducted to characterize the root-knot species occurring in rice fields in Nepal and to study their variability using traditional and molecular tools. The representative Nepalese isolates were also compared to isolates of *M. graminicola* from different countries (India, Bangladesh, and USA). Summaries of the results of this investigation were reported previously (Pokharel et al., 2004; Pokharel et al., 2005).

**Materials and Methods**

*Sample collection and maintenance*

Composite soil samples were collected from rice-wheat fields in Nepal (Figure 1.1) and existing root-knot nematodes (*Meloidogyne spp.*) present in each sample were trapped out on rice cv. BR11. A total of 57 soil samples (10 samples collected in a previous survey from stunted and symptomatic rice plants, along with 47 fresh soil samples collected at random from different fields and geographic locations) were utilized in this investigation conducted at Cornell University. The various nematode collection sites (Figure 1.1) were of different, soil types (heavy or light texture), cropping patterns (rice-wheat vs rice-other crops), samplings criteria (random vs from symptomatic plants) and altitudes [High/mountain (high altitude) vs Tearai (Low altitude)] and river basin (Table 1.1). Soil samples were collected before rice planting or early in the rice season. For random sampling, one composite sample was collected per field (0.1 to 1 ha area) consisting of 10 sub-samples collected in a “W” sampling pattern. These sub-samples were mixed together and a representative 200 cc soil
sample was placed in a labeled plastic container and kept in a cool room for 3-4 weeks before shipping to Cornell University. At Cornell University, samples were mixed with sterile sand placed in 15-cm clay pots, planted to rice cv. BR11 and maintained in a greenhouse at 25 C for 60 days. Plants were then uprooted, washed and scored for infection and severity of root-galling caused by root-knot nematodes. One isolate of *M. graminicola* (BP 3) was obtained from the study of John Padgham (2003) and included for comparison, when possible.

To study the variability within *M. graminicola* isolates from Nepal and other regions, ten isolates of the root-knot nematode were obtained and maintained in greenhouse on the susceptible rice cv. Mansuli. Three isolates from Nepal characterized by Pokharel et al. (2004), 3 isolates from India (provided by Raghab Gupta, a farmer from Madhubani, India), 3 isolates from Bangladesh (Padgham, 2003), and 1 isolate from Florida were used. The isolate from Florida was collected from *Cypreus rondendus* and was supplied by Dr. Janete A. Brito (Florida Dept. of Agriculture and Consumer Services, University of Florida), whereas the other isolates were collected from rice-wheat production fields.

All of the Nepalese soil samples assayed produced galls typical of root-knot nematode infection on rice cv. BR 11, except 4 samples (3 from Dhading and one from Gorkha). The root-galling severity (RGS) rating on rice roots was determined on a 1-9 scale (where 1= no galls and 9=>76% roots with galls) for all 53 samples tested and ranged from 2 to 7. Out of these, 28 root-knot isolates that produced a large number of galls (3-7 RGS ratings) on rice were similarly re-established and maintained for further study. In addition, 5 isolates with RGS ratings of 2 (1-3 galls/root system) were randomly selected and maintained for later use and comparison. A total of 33 isolates were confirmed to cause consistent infections and galls on rice, thus were maintained and used in this investigation. The symptoms
produced by these isolates were similar to the symptoms observed in the field and also described for *M. graminicola* on rice.

The 33 isolates producing distinct galls were cleaned by growing them on the susceptible rice cv. BR11 repeatedly. These isolates were grown for 60 days, uprooted, roots washed free of soil, RGS index determined and eggs were extracted by a modification of the sodium-hypochlorite method (Barker, 1985). The extracted eggs were again used to infest pasteurized soil placed in a 15-cm diameter clay pot and then planted to rice cv. BR11 and maintained at 25 C in the greenhouse for 10 weeks.

Since genetic variation in populations within a field is expected, separation of individuals from within each isolate was essential for further studies. Roots of infected plants were washed, female(s) from a single gall of an isolate was teased out and the egg mass/es were separated by a forceps and used to inoculate rice cv. Mansuli growing in a new pot with sterile soil. Five pots were assayed (5 replications) from each soil sample. Thus, five isolates were established. These isolates were maintained in the greenhouse for further studies and were designated accordingly.

*Morphometric measurements*

For larval measurements, eggs were obtained by blending galled root segments in 1% sodium hypo-chlorite solution, rinsed several times with tap water, and allowed to hatch in tap water for 48 hours. Juveniles were picked in mass randomly, placed in a drop of water on a glass slide and killed by gentle heat. The larvae were covered with a glass cover slip and 35 larvae were selected per isolate and measured. Measurements made included body length, width, stylet length, tail to terminus length.
Table 1.1: Characteristics of sample collection sites of the isolates of root-knot nematodes used in the study.

<table>
<thead>
<tr>
<th>Isolate No</th>
<th>Location</th>
<th>District</th>
<th>Soil type</th>
<th>Previous Sample</th>
<th>Crop type</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP 1</td>
<td>Bhairahawa</td>
<td>Rupandehi (T)</td>
<td>Clay loam</td>
<td>Wheat</td>
<td>Symptomatic</td>
</tr>
<tr>
<td>NP 2</td>
<td>Maghe</td>
<td>Illam (H)</td>
<td>Sandy loam</td>
<td>Fallow-corn</td>
<td>Random</td>
</tr>
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<td>NP 3</td>
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<td>Rupandehi (T)</td>
<td>Loam</td>
<td>Wheat</td>
<td>Symptomatic</td>
</tr>
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<td>NP 8</td>
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<td>Lamjung (H)</td>
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<td>Fallow</td>
<td>Random</td>
</tr>
<tr>
<td>NP 10</td>
<td>Bairahawa</td>
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<td>Clay-loam</td>
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<td>Symptomatic</td>
</tr>
<tr>
<td>NP 12</td>
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<td>Wheat</td>
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<td>Random</td>
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<td>Random</td>
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<tr>
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<td>Sandy-loam</td>
<td>Wheat</td>
<td>Random</td>
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<tr>
<td>NP 28</td>
<td>Budhabare</td>
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<td>Sandy</td>
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</tbody>
</table>
(Table 1.1 continued.)

<table>
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<tr>
<th>NP 38</th>
<th>Madi</th>
<th>Chitwan (T)</th>
<th>Sandy</th>
<th>Wheat</th>
<th>Random</th>
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</thead>
<tbody>
<tr>
<td>NP 39</td>
<td>Bargachi</td>
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<td>Silty-clay</td>
<td>Fallow</td>
<td>Random</td>
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<tr>
<td>NP 40</td>
<td>Aitebare</td>
<td>Illam (H)</td>
<td>Clay-loam</td>
<td>Fallow</td>
<td>Random</td>
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<tr>
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<tr>
<td>NP 43</td>
<td>Paudi</td>
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<td>Random</td>
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<td>NP 44</td>
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<td>Parsa (T)</td>
<td>Sandy-loam</td>
<td>Wheat</td>
<td>Symptomatic</td>
</tr>
<tr>
<td>NP 46</td>
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<td>Chitwan (T)</td>
<td>Sandy</td>
<td>Wheat</td>
<td>Random</td>
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<td>Wheat</td>
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<td>Parsa (T)</td>
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<tr>
<td>NP 55</td>
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<td>Rupandih (T)</td>
<td>Clay-loam</td>
<td>Wheat</td>
<td>Random</td>
</tr>
</tbody>
</table>

a Closest town to sampling site.

b Districts sampled were located the topographic region of Terai (T), Hills (H), River Basin (RB).

c Refers to samples collected from rice fields either randomly or from around symptomatic rice.
Figure 1.1. Sketch map of Nepal showing the collection sites of the Nepalese isolates of root-knot nematodes in Terai (dark), low hills (middle white) and high hills (light brown) production regions. Larger dots refer to areas where higher numbers of samples were collected, whereas fewersamples were collected from areas identified with smaller dots.
and esophagus length. The A value (length/maximum body width), B value (length/esophagus length), and C value (length/tail to terminus) were calculated from the measurements. In addition, 40 larvae from each of the isolates of India, Bangladesh and the USA were measured similarly and compared to selected Nepalese isolates. Means and standard deviations were calculated and compared using Proc GLM (SAS Institute) among the samples used in this study.

*Perineal pattern study*

All the root-knot nematode isolates from Nepal and those from Bangladesh, India and Florida, USA were grown on the susceptible rice cultivar cv. Mansuli in the greenhouse. Mature females were picked-up from the galled roots and placed on a glass slide in a drop of water. The posterior end of females were cut with a sharp razor and cleaned. The glass slide with the female end (cut side down) was covered with a cover-slip and sealed. Ten female perineal patterns were analyzed per isolate. In total, 430 perineal patterns were evaluated in this study. The patterns were examined under a compound microscope at x500 and x640 and selective representative patterns were photographed using Kodak TMAX 100 film.

*Host-range study*

Symptoms produced by all 33 isolates on rice and barnyard grass (*Ehinocloa crusgalli*), a common weed in rice fields, were recorded. Isolate NP 29 was selected as a representative isolate and used to inoculate plants with a known reaction to *M. graminicola*. Reproduction of this nematode isolate and an isolate of root-knot nematode from Bangladesh (BP3) was determined on the North Carolina host range test.
as well as on the following crops; E. crusgali, Jute (cvs. Tosa and Deshi); cabbage cv. Dawith Green; wheat (different varieties); tomato cultivars Rutgars, Money Maker and Cherry Large Red; oat; barley; corn; and rye (Appendix Table 1.2). The North Carolina host range test consisted of tobacco cv. NC 95, watermelon cv. Charlestone Grey, cotton cv. Deltapine 61, pepper cv. California Wonder, tomato cv. Rutgers and peanut cv. Florunner. The seeds of these plants were grown in pots filled with pasteurized soil infested with one of the isolates. Similarly, rice cv. Mansuli, wheat cv. Achyut, Jute cvs. Deshi and Tosa, Oat, barnyard grass and tomato (Red Cherry type, Money Maker and Rutgers) were inoculated with M. graminis for comparison (Appendix Table 1.2 and 1.3). All host range experiments were conducted at an infestation level of 10 eggs/cc soil (10-cm diameter pot) in a greenhouse at 25 C and replicated 5 times. All observations were determined 75 days after planting and all the experiments were repeated once.

To understand the pathogenetic ability of the isolates from different countries, pathogenicity of 4 isolates (one each from Nepal, Bangladesh, India and Florida) of M. graminicola was determined on barnyard grass (E. crusgali), Jute cvs. Tosa and Deshi; Rice cvs. Cordie and Rampur Mansuli; wheat cvs. Harus and Brikuti and tomato cvs. Rutgers, Money Maker, Large Red Cherry (Appendix Table 1.3). Host crops used in the North Carolina host range test were also included in evaluating these four isolates.

Variability in the aggressiveness of the isolates

Virulence is the ability of the nematode isolate to attack and reproduce on a resistant host, whereas aggressiveness is the ability of the nematode isolate to attack and reproduced on a susceptible host (Hussay and Janseen, 2002). Yik and Birchfield, (1979) reported rice cv. LA 110 to be the most resistant and cv. Labelle as one of the
13 most susceptible varieties to a Lousinia isolate of *M. graminicola*. However, a preliminary screening test of commonly grown Nepalese rice varieties to a Nepalese isolate of *M. graminicola* (10 eggs/cc soil) indicated susceptibility of all commercial rice varieties, including the resistant germplasm (LA 110). Thus, root-knot isolates were categorized based on their level of aggressiveness (reaction on susceptible rice varieties). The same rice varieties used to differentiate the aggressiveness of Nepalese isolates were also used to differentiate variability in aggressiveness of isolates from different geographic regions in a separate experiment. Plants of these two varieties of rice (LA 110 and Labelle) were inoculated with the 33 isolates from Nepal and 10 isolates of *M. graminicola* collected from different geographic regions in separate experiments. These plants were maintained in a greenhouse at 25°C for 60 days.

After 60 days, plants were removed from pots and the roots were washed clean of soil. Nematode eggs were extracted by blending for 3 minutes intermittently in 1% sodium hypo-chlorite solution. The contents from the blender were emptied into a #100 sieve nested on top of a #500 mesh and washed for 3 minutes with tap water. Eggs were transferred into a beaker, volume adjusted, and eggs in a 10 ml aliquot were counted under a dissecting microscope. A reproductive factor, \[ RF = \frac{\text{total number of eggs and juveniles extracted}}{5,000} \] (the number of eggs used to infest the soil into the pot), was calculated for each nematode isolate on each differential crop included in the test. The most, intermediate and least aggressive or virulent isolates were identified based on the calculated RF values. All the experiments were repeated once and the results of repeated experiments are presented in appendix Tables.
Molecular characterization of isolates from Nepal using the internal transcribed spacer (ITS) region sequence.

As no ITS sequence data for *M. graminicola* was available in GenBank, a known *M. graminicola* isolate obtained from naturally infected nut-sedge in Florida, USA (described above) was also included in the study. Sequence data for *M. graminis* was also not available in GenBank, thus a known *M. graminis* isolate obtained from turf grass near Buffalo, NY (provided by Dr. Nathaniel Mitkowski, University of Rhode Island, Kingston, RI) was also included in this study. Primers rDNA2 (5’-TTGATTACGTCC-CTGCCCTTT-3’) and rDNA1.58s (5’-ACGAGCCCGA-GTGATCC-ACCG-3’) (Power, 2004) obtained from Sigma Genosys Inc. (St. Louis, MO 63178) were used in this investigation. Established isolates derived from eggs recovered from a single gall, most likely infected by a single female, of the original root-knot nematode isolates from Nepal were utilized. Eggs were obtained by the sodium hypo-chlorite method, allowed to hatch for 3 days at room temperature, and then 60 larvae were picked, rinsed 3 times with sterile distilled water, crushed in 60 µl sterile distilled water on a sterile glass slide, and placed in a small micro centrifuge tube to be used as DNA template. Each DNA template was distributed into five tubes containing 12µl of template/tube and frozen until PCR was performed. A master mix (mm) of 16.25µl of water, 1.5µl of 25 mM MgCl₂, 2.5µl of magnesium free buffer, 0.75 µl of 200mM dNTPs and 1.5 µl of 10 mM of each primer per reaction tube was prepared. Twenty-four µl of master mix (mm) was placed into a micro centrifuge tube and 12 µl of DNA template was added (Mitkowski et al., 2003). Light mineral oil was then added to 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 Corp.) was added to each tube through mineral oil. The PCR
cycle consisted of an initial step at 94 C for 2 minutes followed by 25 cycles of 24 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. Amplicons were visualized by gel electrophoresis. The remaining PCR product was purified with the Promega DNA cleaning kit (Promega Co., Madison, WI) and used for direct sequencing in both directions using the same amplification primers (Sigma Genosys, USA). The ITS sequences of the other Meloidogyne species included in the comparison were obtained from GenBank (acc. No. AY593889 for M. chitwoodi, acc. no. 26892 for M. javonica, acc., AY 53899 for M. minor, acc. Ay 438556 for M. incognita, acc. AF 387092 for M. aranaria, acc. AF 576722 for M. hapla, acc. AY 59301 for M. naasi and acc. AY 07709 for M. trifoliophila). The computer program Infomax 2003 (Infomax, Bethesda, Maryland) was used for alignments of sequences. For amplification products that could not be sequenced directly, PCR products were cloned using the TA cloning kit (Invetrogen, Carlsbad, CA) according to manufactures instructions. Those cloned segments were sequenced using universal forward and reverse primers. Genetic distances among the taxa and isolates were evaluated by three independent phylogenetic analyses using PAUP 4.0 (Swofford, 2001). Unweighted maximum parsimony analysis of alignments was performed using heuristic search. Gaps were treated as missing data. Bootstrap analysis with 1,000 replicates was conducted to assess the degree of support for each branch on the tree. Similarly, ITS sequences of all other isolates were used in comparing the variability of isolates from different geographic regions.
Results

Morphometric measurements of larvae

Average body length, stylet length and A, B and C values of all isolates examined from Nepal were 450.9 (425-477) µm, 11.37 (9.6-15.9) µm, 25.8 (22.3-30.2) µm, 3.8 (3.5-4.2) µm and 6.4 (5.2-8.1) µm, respectively. The average measurements recorded for the 35 individuals per isolate including the known isolate of *M. graminicola* from Bangladesh and US are given in Table 1.2. Although measurements varied among the Nepalese isolates they were generally within the range of measurements described for *M. graminicola*. However, individuals in some of the Nepalese isolates were beyond the range described for *M. graminicola* in the literature (Mulk, 1976). The larval measurements of the Nepalese isolates differed significantly from those of the Bangladesh and US isolates. The Bangladesh isolate was significantly longer, whereas the US isolate was significantly shorter than the Nepalese isolates (Appendix Table 1.2). A significant correlation (P = 0.0025) was observed only between stylet length and body length.

Similarly, high variability was observed in total body length of larvae of isolates from Bangladesh, India, Nepal and USA ranging from 395 to 585, 390 to 500, 373 to 510 and 375 to 470 µm, respectively (Appendix Table 1.3). Variability in esophageal length and the A, B and C values were also observed among these isolates. However, there was no correlation between the various parameters measured and the geographic origin. For example, one of the isolates from Bangladesh was significantly the longest in body length only, (Appendix Table 1.1), whereas the other two isolates from Bangladesh included in this test were not.
**Characterization and variability of perineal patterns**

The perineal patterns of the Nepalese isolates were dorso-ventral, oval to almost circular in shape, moderate in height of arc, and no lateral incisures or gaps were observed. Tail tip was marked with prominent, coarse, fairly well separated striae that sometimes forming an irregular tail whorl. These perineal patterns were similar to the pattern described for *M. graminicola*, with some minor variations (circular markings in the dome of perineum) that resembles those of *M. oryzae* and *M. trifoliophila*. Four variant types (difference in markings on the dome of perineum) in the perennial patterns were recognized (Figure 1.2), although they all resembled those of *M. graminicola*. The perineal patterns of the 10 isolates included in the study of variability among *M. graminicola* from different countries were similar to those observed above among the Nepalese isolates.

**Infection, symptoms and host range studies**

In rice, all the root-knot isolates examined caused swelling and galls of different shape and size throughout the root system. In addition to hook-shaped root tips, infected plants produced numerous small fibrous roots and by-forked roots (Figure 1.3). However, such symptoms were not consistent with the root-knot isolate, but rather were related to the reaction of the rice germplasm as the susceptible varieties exhibited larger number of bigger galls. The initial white root galls became dark brown and necrotic at plant maturity. However, infected roots of older plants always yielded larger numbers of eggs and larvae, regardless of the number and size of galls observed. Eggs were laid within the root cortex and without easily observable egg sac. Higher severity of infection and large white spongy root galls were observed
on *Ehinocea crusgali*, which also had the higher RF values than those calculated for the susceptible rice cultivar Mansuli.

The Nepali (NP 29) and the Bangladesh (BP 3) isolates did not multiply on any of the hosts included in the North Carolina host-range test in repeated experiments. Similarly, these same isolates did not multiply in tomato cvs. Rutgers, Money Maker and Red Cherry type as well as jute cv. Tosa, but they multiplied in cabbage cv. Dawith Green and jute cv. Deshi. In addition, they multiplied in rice, wheat, oat and barley; but not in rye and corn. The reproductive factor of the Nepalese isolate was lower in cabbage and oat as compared to rice, wheat and *E. crusgali*. *M. graminis* multiplied well in *E. crusgali*, but poorly in tomato cv. Rutgers, rice cv. Mansuli and jute cv. Deshi (Data for the host range is presented in Table 1.3).

Variability in aggressiveness of the isolates

Both the rice varieties (Labelle and LA110) tested were susceptible to Nepalese isolates of *M. graminicola*. Thus, these isolates were considered aggressive on both varieties. However, galling severity and RF values varied in both rice varieties among the Nepalese root-knot isolates. Based on the results obtained, isolate NP 37 and NP 36 were the most aggressive, whereas isolate NP 39 was the least aggressive. The isolates could be classified into 3 categories based on the results of aggressiveness tests. The most aggressive, the intermediate and the least aggressive isolates. Four isolates (NP 37, NP 12, NP 36 and NP 50) were in the most aggressive category and four isolates (NP 49, NP 40, NP 46 and NP 29) were categorized as the least aggressive. The remaining isolates were categorized as intermediate types in their aggressiveness to these rice varieties (Table 1.4). There was no correlation among the aggressiveness of the Nepalese isolates and the geographic areas of collection, soil
Table 1.2: Morphometric measurements (µm) of larvae of the Nepalese isolates, on rice cv. BR 11 in the greenhouse.

<table>
<thead>
<tr>
<th>Isolates No.</th>
<th>Body length</th>
<th>Stylet length</th>
<th>A value(^a)</th>
<th>B value(^b)</th>
<th>C value(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP 1</td>
<td>438.3 klm</td>
<td>10.1 ab</td>
<td>25.5 fghi</td>
<td>3.65 bcde</td>
<td>6.16 ghijk</td>
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<tr>
<td>NP 2</td>
<td>431.6 lm</td>
<td>12.9 d</td>
<td>22.9 lm</td>
<td>3.9 efgh</td>
<td>6.29 efghi</td>
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<tr>
<td>NP 3</td>
<td>448.8 efg</td>
<td>11.4 cd</td>
<td>22.32 m</td>
<td>3.87 defgh</td>
<td>6.11jklm</td>
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<tr>
<td>NP 8</td>
<td>463.2 bcd</td>
<td>09.77 ab</td>
<td>27.43 abcd</td>
<td>4.26 i</td>
<td>6.56 abcde</td>
</tr>
<tr>
<td>NP 12</td>
<td>456.5 bcde</td>
<td>11.85 cd</td>
<td>26.4 cdefg</td>
<td>4.12 hi</td>
<td>8.11 a</td>
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<td>NP 10</td>
<td>477.0 ab</td>
<td>11.95 cd</td>
<td>25.81 efg</td>
<td>3.88 defgh</td>
<td>6.82 abcd</td>
</tr>
<tr>
<td>NP 16</td>
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<td>11.17 cd</td>
<td>24.33 hiijk</td>
<td>3.73 cdef</td>
<td>8.13 a</td>
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<tr>
<td>NP 20</td>
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<td>11.46 cd</td>
<td>25.37 ghi</td>
<td>3.93 efg</td>
<td>7.28 a</td>
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<td>NP 22</td>
<td>456.6 bcde</td>
<td>11.45 cd</td>
<td>26.2 cdefgh</td>
<td>3.72 cdef</td>
<td>6.51 bcde</td>
</tr>
<tr>
<td>NP 24</td>
<td>437.4 lm</td>
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<td>23.71 ijk</td>
<td>3.78 cdefg</td>
<td>6.31 efg</td>
</tr>
<tr>
<td>NP 25</td>
<td>462.7 bcd</td>
<td>11.91 cd</td>
<td>24.93 ghi</td>
<td>3.99 ghi</td>
<td>6.12 ijk</td>
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<td>446.4 efg</td>
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<td>25.81 efg</td>
<td>3.78 cdefg</td>
<td>6.82 abcd</td>
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<td>NP 29</td>
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<td>3.77 cdefg</td>
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<td>4.03 hi</td>
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<td>25.44 ghi</td>
<td>3.84 cdefg</td>
<td>6.28 fghi</td>
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<td>NP 35</td>
<td>443.6 hij</td>
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<td>26.95 bcdef</td>
<td>3.75 cdefg</td>
<td>6.11 klm</td>
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<td>461.2 bcde</td>
<td>11.52 cd</td>
<td>27.58 abcd</td>
<td>3.68 bcdef</td>
<td>6.88 abc</td>
</tr>
<tr>
<td>NP 37</td>
<td>473.6 ab</td>
<td>11.48 cd</td>
<td>25.83 efg</td>
<td>3.82 cdefg</td>
<td>7.02 ab</td>
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(Table 1.2 continued)

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<td>11.2 cd</td>
<td>26.17 efg</td>
<td>3.58 bcd</td>
<td>6.65 abcde</td>
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<td>425.2 n</td>
<td>10.92 c</td>
<td>28.5 abc</td>
<td>3.62 bcd</td>
<td>6.32 defgh</td>
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<td>454.2 bcd</td>
<td>11.26 cd</td>
<td>29.84 abc</td>
<td>3.91 efg</td>
<td>6.40 cdefg</td>
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<td>442.0 hij</td>
<td>11.19 cd</td>
<td>24.79 hijk</td>
<td>3.69 bcd</td>
<td>6.54 bcde</td>
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<tr>
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<td>430.0 m</td>
<td>11.28 cd</td>
<td>23.53 jkl</td>
<td>3.69 bcd</td>
<td>6.51 bcdefg</td>
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<td>10.14 ab</td>
<td>30.26 a</td>
<td>3.87 defg</td>
<td>6.07 lmn</td>
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<td>467.2 abc</td>
<td>11.41 cd</td>
<td>26.83 bcd</td>
<td>3.89 defgh</td>
<td>6.66 abcd</td>
</tr>
<tr>
<td>NP 46</td>
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<td>11.47 cd</td>
<td>23.76 jkl</td>
<td>3.57 ab</td>
<td>6.42 cdefg</td>
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<td>449.3 efg</td>
<td>11.25 cd</td>
<td>25.56 fghi</td>
<td>3.87 defgh</td>
<td>6.38 defgh</td>
</tr>
<tr>
<td>NP 50</td>
<td>466.0 abc</td>
<td>11.44 cd</td>
<td>26.39 cdefg</td>
<td>3.86 cdefg</td>
<td>6.36 defgh</td>
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<td>11.30 cd</td>
<td>27.02 bcd</td>
<td>3.70 bcd</td>
<td>6.58 abcde</td>
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<td>BP3</td>
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<td>4.02 ghi</td>
<td>6.31 efg</td>
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<td>US 1</td>
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<td>10.28 b</td>
<td>29.92 ab</td>
<td>3.42 a</td>
<td>5.87 mn</td>
</tr>
</tbody>
</table>

The means followed by the same letters in a column are not significantly different by Tukey’s test (P=0.05 level).

\(^a\) Refers to the ratio of body length/maximum body width.
\(^b\) Refers to the ratio of total body length/neck length.
\(^c\) Refers to the ratio of total body length/tail length.
Table 1.3: An expanded North Carolina host range tests, for differentiating species and races of *Meloidogyne*.

<table>
<thead>
<tr>
<th>Meloidogyne Spp. or Races</th>
<th>Tobacco Water Melon</th>
<th>Cotton Pepper</th>
<th>Tomato</th>
<th>Peanut</th>
<th>Wheat</th>
<th>Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. incognita</em> Race 1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>M. incognita</em> Race 2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>M. incognita</em> Race 3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>M. incognita</em> Race 4</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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a) The cultivars of tobacco, water melon, cotton, pepper, tomato, peanut, wheat and rice etc used were NC 95, Charleston Grey, Delta Pines, California Wonder, Rutgers, Florunner, Nuhaines and Mansuli.

b) *F* Florida isolate and *O* others (Isolates from Nepal, India and Bangladesh).
Table 1. 4: Root galling severity (RGS) ratings and reproductive factor (RF) resulting from inoculating roots of rice cv. Labelle and cv. LA 110 with *M. graminicola* isolates from Nepal.

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<th>Cv. Labelle RF(^b)</th>
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Means in a column followed by the same letter are not significantly different by Tukey’s test (P=0.05 level).

a Root gall severity ratings were recorded on a scale of 1 (healthy roots, no galls observed) to 9 (>76% of roots with galls).

b Refers to reproductive factor (RF=Pf/Pi).

c The rice cultivars Labelle and LA 110 were reported as most susceptible and resistant, respectively to isolates of *M. graminicola* in Lousinia, USA (Yit and Birchfield, 1979).
Figure 1.2. Variability in the perineal pattern observed among the Nepalese root-knot isolates, showing minor variants in marking (punctuation) identified.
Figure 1.3. Different shape and size of galls, extensive branching, and root tip thickenings caused by root-knot nematode infections in rice.
types or crop rotation practiced. Similar results were observed when the experiment was repeated. In addition, significant differences in root-galling severity and reproductive factors among the isolates of *M. graminicola* from India, Bangladesh and Nepal were also observed on rice cvs Labelle and LA 110. The Florida isolate induced the lowest RGS and RF values in both varieties Isolates B2 and N3 produced the highest root-galling severity on LA 110 and Labelle, respectively. Similarly, I2 and N3 produced significantly the highest RF values on LA 110 and Labelle, respectively (Appendix Table 1.3)

*Molecular characterization of isolates by internally transcribed spacer (ITS) Region.*

Since the morphometric measurements, perineal pattern, and root-galling symptomology were generally similar to those of *M. graminicola*, identity and variability of the Nepalese isolates were further confirmed by molecular characterization. Amplification and sequencing of the ITS regions of the root-knot nematodes collected from rice-wheat production systems in Nepal yielded a single fragment of approximately 450 nucleotides (Appendix Table 1.3). The ITS sequence of each of the 29 Nepalese isolates was obtained and GenBank accession numbers are listed in Table 1.5. All Nepalese isolates formed a single clade distinctly different than all other common species of *Meloidogyne*, including the closely related *M. graminis*. Nucleotide polymorphism was observed among many isolates (Appendix Figure 1.3), but only within a few isolates. The nucleotide polymorphism variant groups were not consistent in nucleotide substitution pattern except for two distinct groups, one representing the hill region isolates and the other representing the Terai region isolates. Genomic DNAs were extracted and preserved for further research.
The parsimony analysis resulted in 8 equally parsimonious trees, differing slightly in their branching pattern. Thus, a strict consensus of all 8 parsimonious trees was obtained and is provided in Figure 1.4. Neighbor-joining resulted into a tree showing a distinct clade of Nepalese, US and Bangladesh isolates, whereas all other Meloidogyne species formed distinct and different clades. Analysis of the nucleotides by neighbor joining clearly showed that the Nepalese isolates formed two distinct haplotypes. One of the haplotypes included all the isolates collected from the Terai region as well as *M. graminicola* isolates tested from US and Bangladesh. All the Nepalese isolates collected from the hill region and *M. trifoliophila*, the species of root-knot reported from white clover, formed the other haplotype. Isolates from the Terai group had relatively fewer variations in nucleotide, whereas isolates from the hill group had higher variability within the ITS region. Isolates of *M. graminicola* from the hill region had a haplotype with CATA at 277 to 280 bp and T, TT or TTT at 447 to 449 bp, whereas the haplotype of the Terai region haplotype had TATT and gaps in the same positions. The bootstrap value (as shown in Figure 1.4 generated by 10,000 replications showed a high degree of significance with all branches supported at the 50% or greater level and some branches with higher than 85% bootstrap value. The bootstrap value for isolates of *M. graminicola* was low as compared to other common *Meloidogyne* species.

The ITS sequences exhibited considerable variation among the 10 isolates of *M. graminicola* examined from different countries. However, the nucleotide alignment exhibited no definitive patterns of variation as random differences in nucleotide substitutions were observed in only a few base pairs among the isolates from within each geographic region. Phylogenetic analysis showed that all *M. graminicola* isolates formed the same clade along with *M. trifoliophila* (Figure 1.4). The other root-knot species included
Table 1.5: GeneBank Accession numbers for the ITS sequences of isolates of *M. graminicola* collected from Nepal and submitted to GenBank.

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The isolates with multiple copies of ITS sequences are differentiated by the decimal point in isolate number.
Figure 1.4. Phylogenetic tree of Nepalese, Bangladesh and US isolates of root-knot nematodes (*M. graminicola*, *M. graminis* and other *Meloidogyne* species) based on ITS sequences.
were distinctly different in sequence alignment as well as in phylogenetic analysis of
the ITS region. The species *M. graminis* and *M. nasii* were most closely related (Fig
1.4).

**Discussion**

Results of this investigation suggested that only *Meloidogyne graminicola*
occurs in the rice-wheat production fields in Nepal. The latter finding was confirmed
by morphometric measurements of larvae, perennial pattern of females, host range
tests, and amplification and sequencing of the ITS regions.

The results also documented the existence of considerable variability among
the Nepalese isolates of *M. graminicola* as well as isolates of this species from India,
Bangladesh and the USA in larval size, host range, aggressiveness on rice germplasm,
and ITS sequences. Several *Meloidogyne* spp. (*M. artiellia, M. chitwoodi, M. naasi, M.*
*microtyla* and *M. ottersoni* (Sikora, 1988), *M. graminicola, M. graminis, M. kikuyensis*
and *M. spartinae* (Taylor and Sasser, 1978) and *M. incognita, M. javanica* and *M.*
*arenaria* (Swarup and Sosa-Moss, 1990)) were reported to attack cereals including
wheat worldwide. However, only *M. graminicola, M. tritocoryzae, M. oryzae, M.*
*incognita, M. javanica* and *M. arenaria* were reported attacking rice. Mostly, *M.*
*graminicola* has been reported on rice from lowland production condition (with short
term flooding and water not more than 10-cm) (for extended period) of time which is
common in most of the Terai regions of Nepal.

The average values for larval measurements of individuals among the Nepalese
isolates falling beyond the range described for *M. graminicola* in literature may be due
to the high variability of this nematode in Nepal and other countries influenced by the
diversity in geographic locations, cropping patterns, soil types and management
options of collected isolates. A total of 1155 individuals were measured in the present study whereas such measurements were generally based on 20-25 individuals in the previous reports. However, there was no correlation between the variability in larval measurements with that of geographic regions. Lower and higher ranges of measurements observed in this study suggested either that the larval measurements are not reliable means of identification of this species due to high variability or they may need readjustment to account for the observed variability. Similar variability in the measurements of *Meloidogyne* larvae was described by Handoo et al. (2003) while describing *M. thailandica*, a new species of root-knot of ginger from Thailand.

Perineal pattern morphology has been the traditional standard tool for the identification of the most common *Meloidogyne* species since 1949 (Chitwoodi, 1949; Taylor et al., 1955). The perineal patterns of the Nepalese isolates in this study were consistent and similar with published perineal patterns for *M. graminicola*, with some minor variability. Variability in perineal patterns was observed, but not in all isolates as 19 isolates had only single type of patterns of the four minor variants observed. The remaining 17 Nepalese isolates examined exhibited mixtures of perineal pattern variants ranging from 2 to 4 variants per isolate. However, the perennial patterns of these isolates were distinctly separable from other common species of *Meloidogyne* including *M. incognita, M. arenaria, M. javonica* and *M. hapla*. In addition, none of the perennial patterns of the examined samples were similar to the perennial patterns described for *M. graminis*, as the lateral incisures distinct for the latter species were absent. *M. graminis* is considered as the closest species to *M. graminicola*, having similar cereal hosts and was expected to occur on wheat and other grain crops. Examination and comparison of the perennial pattern variants observed on the collected Nepalese isolates suggested some overlap with those reported for *M. oryzae* and *M. trifoliophila*. *M. oryzae* has been reported on rice only once from Surinam.
(Maas et al., 1978). However, the possible existence of *M. oryzae* with *M. graminicola* in rice fields can’t be ignored. The root-knot species *M. trifoliophila* was reported only from white clover and is not known to attack rice or wheat (Bernard and Eisenback, 1997). The minor variation observed in the perineal pattern of the Nepalese isolates could be of natural occurrence within the species caused primarily by difference in size of females examined or due to variation in genetic make-up of the species.

Variability in the perineal pattern among the isolates of *M. graminicola* collected from different countries were similar to those observed among the Nepalese isolates, but only 3 of the 4 minor variants were observed. Variations in perineal pattern within species have been described in other species of *Meloidogyne* (Jepson, 1985; Mitkowski et al., 2003; Handoo et al., 2005). In addition, minor variation (markings on perineum) in perineal pattern of *M. graminicola* was described in the original description of the species (Mulk, 1976). Nevertheless, the perennial patterns alone could not be used to confirm the identity of root-knot nematode isolates from Nepal, as the perennial patterns for *M. oryzae*, *M. trifoliophila* and *M. graminicola* are not unique enough for making a definitive diagnosis.

Root-knot nematode infections on lowland rice resulting in the production of hooked-like galls on roots have been attributed to *M. graminicola* or possibly *M. oryzae* (Luc et al., 1990). Also, it was reported that *M. graminicola* and *M. oryzae* are the only root-knot species that can survive in flooded soil conditions (Bridge et al., 1990). *M. oryzae* has been reported only once from Surinam and can survive flooding for short period at a depth of less than 10-cm water, thus such characteristic root gall symptoms (the hook-like galls) in rice are considered diagnostic for infection caused primarily by *M. graminicola*. Similar symptoms on rice roots infected with the Nepalese root-knot isolates were observed on rice in the greenhouse in this study.
In this study, numerous small roots, root proliferation, by-forked roots and swelling of root-tips in addition to hook-like galls (Figure 1.3) were observed on rice plants infected with the Nepalese isolates of *M. graminicola*. These symptoms are also produced by other species of *Meloidogyne*, except the hook-like galls. Egg sacs were not observed on the surface of rice roots as eggs were deposited in the root cortex in mass, unlike other species of *Meloidogyne*. Failure to observe egg sacs in rice roots infected by the Nepalese isolates also suggested their identity as *M. graminicola* (Mulk, 1976; Reversat and Fernandez, 2004).

All the Nepalese isolates of *M. graminicola* and isolates from different countries included in this study infected and multiplied well in barnyard grass in a similar manner to the *M. graminis* isolate collected from grasses as described by Birchfield (1965). Also, the isolates tested reproduced in rice in contrast to *M. graminis*. These results also indicated that barnyard grass can serve as an experimental differentiating host for both species. Fluffy, large and white galls without a hook were observed in barnyard grass. However, infected roots turned brown and the galls were difficult to recognize at plant maturity as was also the case in older and infected rice roots. Eggs and larvae were always observed and recovered from such infected roots. In our previous field surveys, large numbers of eggs and juveniles were recovered from brown roots without observable galls. In most greenhouse tests, infected roots became brown in color as the plants reached maturity and galls were generally not visible on such infected roots. At plant maturity, rice roots turn brown due to secondary infections and/or due to physiological changes. Similar observations were made by Birchfield (1965) in *E. colunum*. A number of isolates of root-knot nematode from Nepal, Bangladesh and Florida (USA) reproduced in jute cv. Deshi, but not in jute cv. Tosa. Padhgam (2003) also reported similar results with *M. graminicola* isolates from Bangladesh. The Nepalese root-knot isolates also reproduced on other
crops such as cabbage, wheat and barley. Thus, cabbage, wheat and barley are good
hosts for *M. graminicola*, whereas corn and oat are not. However, oat and corn were
previously reported as hosts to *M. graminicola* (MacGowan and Langdon, 1989). This
contradicting result may be due to the isolates of *M. graminicola* and the varieties used
in the study of MacGowan and Langdon (1989) and those used in the current
investigation.

Isolates of root-knot nematode from Nepal, Bangladesh and Florida did not
multiply in any of the plants included in the North Carolina host range test, namely
tobacco cv. NC 95, watermelon cv. Charlestone Grey, cotton cv. Deltapine 61, pepper
cv. California Wonder, tomato cv. Rutgers and peanut cv. Florunner. Moreover,
reaction of these host plants to *M. graminicola* was not known and the results obtained
indicated that the current make-up of the North Carolina host range test is not suitable
for differentiation of *M. graminicola*. Tomato cv. Rutgers is an experimental host for
most of the root-knot species, but not the root-knot species attacking cereals, like *M.
graminicola*, *M. oryzea* and *M. graminis*. However, *M. oryzea* multiplied well in
tomato cv. Money Maker (Maas et al., 1978), but *M. graminicola* did not multiply in
tomato cv. Rutgers (Brachfield, 1965) or Large Red Cherry type (Manser, 1971).
Nepalese isolates with observed overlapping perineal patterns to those of *M. oryzea*
were tested and found that these isolates did not multiply in any of the above tomato
varieties. This finding indicated that these isolates were not *M. oryzae*. However, all
the Nepalese isolates were not tested on these three tomato varieties.

The rice cv. Labelle and cv. LA 110 were previously reported to be susceptible
and resistant, respectively to an isolate of *M. graminicola* from the Louisiana, USA
(Yik and Brichfield, 1979). However, both varieties were susceptible to the Nepalese
isolates of root-knot (high root-galling severity ratings and high RF values) in this
study. The difference in results obtained may be due to the high virulence of isolates
used in the present study or the different methods employed. In the study of Yik and Brachfield, only RGS ratings were used to differentiate resistance and susceptibility of the varieties, whereas both RGS ratings and reproduction of the nematode isolates were used in the present study. In addition, they used an isolate from Lousinia, USA where rice is not commonly grown, whereas the isolates from Nepal used in the present study were obtained from rice, which is extensively cultivated year after year in the same field.

Significant variability in aggressiveness was also observed among the 10 isolates of *M. graminicola* from different countries included in this study. All the isolates tested from Nepal, Bangladesh and India were highly aggressive on the rice cultivars tested (cvs. Mansuli, Labelle, LA 110 and Cordie), whereas the Florida (US) isolate was not. However, all isolates were highly aggressive on the wheat cultivars tested.

The intra-species variation in *Meloidogyne* spp. was reported to be closely related to the pathogeneicity and aggressiveness of respective species or races (Baicheva et al., 2002). In the present study, distinct intra-specific variability within the *M. graminicola* species was observed leading to differentiation of two distinct pathogenicity groups. Thus, *M. graminicola* can be categorized into two distinct races or biotypes as described by Triantaphyll (1984) and Van der Beek, et al. (1999). Similar variability was documented within other species of *Meloidogyne* including *M. chitwoodi*, *M. incognita*, *M. arenaria* resulting in the recognition of races within each species (Sasser, 1979; Griffin and McKenry. 1989; Santo and Pinkerton, 1985).

To our knowledge, this is the first report of ITS sequences for *M. graminis* and *M. graminicola*. A BLAST search revealed that the ITS sequences of *M. graminicola* were very similar to the sequences of *M. trifoliophila*. It is possible that these species have similar ITS sequences and/or are the same species or sub-species. *M. trifoliophila*
has similar larval measurements and overlapping perineal patterns to those of *M. graminicola*. ITS sequence data from *M. graminis* and *M. graminicola* were very distinct and easily separable from each other and other species, except *M. trifoliophila*. Unfortunately, we did not have an isolate of *M. trifoliophila* to include in the test. *M. trifoliophila* was observed infecting clover, but it has not been shown to infect rice. Initially, the root-knot nematodes infecting clover and other related plants were considered to be *M. graminicola*, but Bernard and Eisenback (1997) recognized them as *M. trifoliophila* based on morphometric measurements, especially the larval tail tip, and also by their different host range. They stated that *M. trifoliophila* rarely parasitized most of the grasses that are hosts to *M. graminicola* and it did not establish itself on rice, wheat, or barley. In addition, it was reported that a USA isolate of *M. graminicola* from rice was also pathogenic to clover (Windham and Penderson, 1992). In New Zealand, Mercker et al. (1997) reported that *M. trifoliophila* was morphologically similar to *M. graminicola*, but they concluded that the dominant root-knot nematode in NZ pastures was not *M. graminicola* because galls were not seen on grasses growing in pastures with heavily galled white clover. However, they did not mention the types of grasses they studied. The Florida isolate of *M. graminicola* and *M. trifoliophila* were similar not only in morphometric measurements and perineal pattern, but also they did not infect and multiply on rice. However, the Florida isolate infected and multiplied well in barnyard grass and wheat cvs. Brikuti and Harus, similar to other isolates of *M. graminicola*.

Parsimony and neighbor joining analysis resulted in a single distinct clade for the Nepalese root-knot isolates and also for both the Bangladesh and the Florida isolates. This clade was well separated from clades of other common species of *Meloidogyne*, with a clear cut number of changes in the nucleotide base pair (which is more than 1%). The Nepalese isolates forming a single clade along with the Florida
and Bangladesh isolates may be due to the fact that ITS sequences of all Nepalese isolates were more similar to *M. graminicola* than any other *Meloidogyne* species. The unique ITS sequences of all Nepalese isolates and *M. graminis* indicated that ITS sequences may serve as identifying tools for separating *M. graminicola* and *M. graminis* from other *Meloidogyne* species. Several studies have demonstrated that ITS was not only useful as diagnostic tool for *Globodera*, *Heterodera*, *Xiphenema*, *Longidorus* and *Pratylenchus* species (Power, 2004), but also for phylogenetic analyses of relationships of a number of species in the genera *Meloidogyne* and *Brusaphelenchus* (Hugall et al., 1999; Power, 2004).

Variability in ITS sequences among the Nepalese isolates and within a few individual isolates was observed. The variability in the ITS sequences indicated that the ITS regions in this *Meloidogyne* species may be useful for population studies. The variability in ITS sequences within an isolate observed in this study could be due to the presence of multiple alleles and/or multiple copies of the sequences which were both reported previously in other root-knot nematode species (Power, 2004). However, ITS sequences and the two distinct ITS haplotypes were not correlated to larval dimensions, perennial pattern, or aggressiveness of the same isolate studied in this investigation. This result is in agreement with the observations made by Hugell et al. (1999) with *M. arenaria*, *M. incognita* and *M. javonica*.

No correlation of nucleotide substitution patterns and identified characteristics of collected isolates including cropping history (rice-wheat vs rice-other rotations), soil texture types (heavy vs light), and sampling designs (random vs symptomatic plants) was observed, except the geographic origin of the isolates. All the Nepalese isolates in clade 1 were collected from rice fields in the Terai (plain) region, whereas
the isolates in clade 2 came from the Hill (high altitude) production region. The Terai and Hill regions stretch out from east to west of Nepal. These geographic regions are dissimilar in their climate and agro-ecological characters.

In summary, this study confirmed that all Nepalese isolates of root-knot nematode collected from rice-wheat were *M. graminicola*. The population is quite homogenous, with only slight variations in morphometric characters, virulence and ITS sequences. Results obtained also suggested the utility of ITS sequences to differentiate *M. graminicola* from *M. graminis* and other common species of root-knot nematode. This information would be useful for further studies on nematode biology, genetics and management. Variability in larval measurement, aggressiveness and ITS sequences was observed among the 10 isolates obtained from rice production areas of different countries (Nepal, India, Bangladesh and USA). This result suggested the existence of at least two races in *Meloidogyne graminicola* that can be separated by rice and wheat differentials. Geographic isolates from Nepal, India and Bangladesh were pathogenic in rice and wheat whereas the isolate from Florida, was pathogenic only on wheat.
Literature cited


CHAPTER 2

PROTOCOL FOR SCREENING RICE AND WHEAT GERMPLASMS FOR RESISTANCE TO MELOIDOGYNE GRAMINICOLA IN GREENHOUSE

Abstract

Root-knot nematode (*Meloidogyne graminicola*) is an important pathogen of rice and wheat grown in South Asia. Greenhouse experiments were conducted to determine the effect of incubation time, initial inoculum density, age of seedlings, inoculation methods and size of planting container on the infection and reproduction of *M. graminicola* in rice and wheat, thereby optimizing a protocol for germplasm screening for resistance to this nematode. Clay pots were filled with pasteurized mineral soil, planted with 10 seeds or seedlings of rice or wheat, inoculated with eggs of *M. graminicola* (0, 100, 500, 1000, 5000, or 10,000 eggs/pot) and were incubated for 45 or 60 days on greenhouse benches at 25° C with daily watering and weekly fertilization. At the end of the incubation period, plants were uprooted, roots washed free of soil, root-galling severity (RGS) ratings determined, eggs extracted from the roots and reproductive factor (RF) was calculated. Higher RGS ratings and the calculated RF values were observed at 60 days of incubation. Also, a significantly higher RF values were observed with lower initial inoculum densities (1000 eggs/pot) at 60 days of incubation. Seeds inoculated with eggs of *M. graminicola* had higher RGS ratings, but lower RF values as compared to seedling inoculations. The RF values were increased with increasing initial inoculum densities upto 2 eggs/ cc soils (1000 eggs/pot) then decreased with higher densities. Inoculated plants growing in clay pots (10-cm in diameter) exhibited higher RGS, but those growing in plastic

*This chapter will be submitted to Journal of Nematology for publication.*

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tubes (100 cc) had higher RF values. The inoculation methods evaluated (mixing eggs suspension with soil, spreading egg suspension on the soil surface or placing eggs suspension in holes around seeds or seedlings) had little effect on RGS rating and RF value. However, mixing inoculum into the soil resulted in the lower RF values and RGS ratings. The significant interactions obtained among initial inoculum densities, seeds vs. seedlings, age of the seedlings, container size and host crop (rice or wheat) on RGS ratings and/or RF of this nematode indicated a need to considerer these factors together. The results obtained also confirmed that the time of year (summer vs. winter months) affected RGS and reproduction of this nematode in the greenhouse. Higher RF values and/or RGS ratings were observed on rice and wheat grown in summer months. Based on the results of this study, the protocol for screening rice and wheat germplasm for resistance to *M. graminicola* includes inoculation of seeds with 1000 or 5000 eggs/10-cm pot (2-10 eggs/cc soil) and incubating for 60 days in greenhouse at 25 C.

**Introduction**

Root-knot nematode (*Meloidogyne graminicola* Golden and Birchfield) is an important pathogen of rice (*Oryza sativa* L.) and is also becoming important on wheat (*Triticum satvum* L.) in rice-wheat production systems of South-East Asia. This nematode was reported to cause more than 30% yield losses on rice in farmer’s fields (Duxbury, 2002; Padgham, 2003; Sharma-poudyal et al., 2005), but its real impact on wheat yield is not well known. Generally, farmers are not aware of problems caused by this nematode because it does not produce conspicuous aerial symptoms on infected plant, and often the nematode induced symptoms are confused with those of other problems including nutritional disorders. In SE Asia, chemical nematicides are
rarely used for nematode control due to high cost, unavailability, and human health and environmental pollution risks associated with their use.

At present, non-chemical management options against this nematodes are used on a limited scale, although they are effective and recommended. Crop rotation can decrease the potential for substantial yield losses due to this nematode (Luc et al., 1990; Whitehead, 1998), but does not seem feasible because of unavailability of profitable alternative crops to rice. In addition, most of the crops grown in rice-based rotations are host to this nematode. Biological control products may have potential for future use, but have limited use at present (Starr et al., 2002). As a result of the limited research devoted to the screening and breeding for resistance against *M. graminicola* in rice and wheat, resistant varieties of rice or wheat are not available. Thus, effective management of this nematode is difficult to achieve and the occurrence and damage of this nematode has been increasing year after year. Nevertheless, exploring and use of host resistance is a good and sustainable management strategy against this nematode, especially for resource poor farmers. It is also critical to determine the reaction of commercial varieties in current use against this nematode. Rotating susceptible crop varieties with resistant varieties or non-host crops is a known strategy for reducing the initial inoculum and minimizing yield losses.

Researchers working with *M. graminicola* in rice used different approaches and screening methodologies in search of resistance factors. The appropriate screening protocol used to identify nematode resistant breeding lines should be capable to readily and reliably evaluate thousands of genotypes encountered in a breeding program (Boerman and Hussay, 1992). Also, it is useful in the evaluation of advanced breeding lines to obtain quantitative data on egg numbers produced in roots, which gives a better indication of resistance than either root-gall or egg mass determination (Luzzi et al., 1987). There are established protocols available to work with other
root-knot nematodes including *M. aranaria*, *M. incognita*, *M. javonica* and *M. hapla* in soybean, potato, tomato, pepper, lettuce and other crops, but unfortunately only limited information is available on protocol for *M. graminicola* in rice and wheat. Moreover, identification of sources of resistance in rice to *M. graminicola* must be performed under environmental conditions that are favorable to the maximum expression of the damage that this nematode causes (Tangingan et al., 1996). Thus, several greenhouse tests were designed and conducted in this investigation to develop an effective, reliable and efficient protocol for the evaluations of rice and wheat germplasms for resistance to *M. graminicola*.

**Materials and methods**

**General Methodology**

Unless otherwise stated, all the experiments reported in this paper were conducted in a randomized complete block design with 5 replications. A 10-cm (500 cc capacity) clay pot (replicate) was filled with pasteurized mineral soil, planted to 10 seeds of rice cv. Mansuli (Nepali) or wheat (Bhrikuti) inoculated with eggs of *M. graminicola* (Bangladesh isolate 3; Padgham, 2003), covered with a thin layer of sterile sand and maintained in greenhouse at 25 C for 60 days. All pots were watered twice daily and fertilized weekly with 30-30-30 NPK fertilizer. After 60 days, plants were uprooted, washed free of soils and scored for infection severity (root-galling) ratings caused by the root-knot nematode. The root galling severity (RGS) was assessed on a 1-9 scale by estimating proportion of roots galled: 1= no galls (healthy roots), 2 = ≤ 5 % roots galled, 3 = 6-10%, 4 =11-18%, 5 = 19-25%, 6 = 26-50%, 7 = 51-65%, 8=66-75%, and 9= 76 -100% of roots were galled (Mullin et al., 1991). Nematode eggs were
extracted by blending roots for 3 minutes intermittently in 1% sodium hypo-chlorite solution (Barker, 1985). The contents from the blender were emptied into a #100 sieve nested on top of a #500 mesh and washed for 3 minutes with tap water, eggs collected into a beaker, volume adjusted to 100 ml, and eggs in a 10 ml aliquot counted under a dissecting microscope. A reproductive factor, [RF = total number of eggs and juveniles extracted from roots and soil divided by the number of eggs used to infest the soil in the pot], was calculated for each pot (replicate). Collected data were tabulated, normality of distribution checked, appropriate transformation done, means calculated and compared using Proc GLM (SAS Enterprise, SAS Institute) among the treatments in each experiments.

**Incubation Period**

Since the life cycle of this nematode is reported to be relatively longer than other-root knot nematodes, incubation periods of 45 and 60 days were tested to determine the appropriate incubation period for assessing infection and reproduction of this nematode on the susceptible rice cv. Mansuli. The two incubation periods were also combined with two inoculum levels (2 and 10 eggs/cc soil) for assessing their impact on the reaction of rice and wheat varieties. The inoculum was placed in a hole next to seeds or seedlings.

**Initial Inoculum Densities**

Often, levels of initial inoculum play a vital role in infection severity and reproduction of root-knot nematodes in host crops. The role of initial inoculum density of *M. graminicola* on its infection severity and reproduction on rice and wheat was not
known well. Rice plants might become infected either in the nursery seed bed and/or in the main field after transplanting. To understand the role of different levels of initial inoculum on direct-seeded or transplanted rice or wheat seedlings, seeds or seedlings (4 weeks old) were inoculated with 0.2, 1, 2, 10 or 20 eggs of *M. graminicola/cc* soil.

Experimental Unit Size

Availability of resources and greenhouse space often limits the numbers of germplasm that can be evaluated. Small plastic tubes, which have higher space use efficiency than the larger pots have been extensively used in other crop-nematode interactions, but not in rice or wheat-*M. graminicola* interactions. Thus, to find the relative effect and efficiency of different container sizes, two sets of experiments were conducted at the same time on rice and wheat comparing the use of clay pots (10-cm diameter, 500 cc of soil capacity) to plastic tubes (volume of 150 cm$^3$ and 20.6 cm long with holes at the bottom (Ray Leach Single Cell Cone-tainers™ systems, Stuewe and Sons Inc., Corvallis, Oregon, USA) (Figure 2.1). Pasteurized mineral soil was placed in the tubes and clay pots, 3 rice or wheat seeds were planted in each tube or pot, inoculated with 0.5, 1, 2 and 8 eggs of *M. graminicola* per cc of soil in a hole along with seeds, covered with a thin layer of peat moss and soil mixture, and incubated on a greenhouse benches for 60 days.

Age of Seedling at inoculation

Two separate experiments were carried out to study the role of inoculating seeds or seedlings of different ages on RGS ratings and RF values. In the first experiment, either seeds or seedlings (30 days olds) were inoculated by placing egg
Figure 2.1. Evaluation of rice germplasms for resistance to *M. graminicola* in plastic tubes and clay pots in the greenhouse.
suspension of *M. graminicola* (10 eggs/cc soil) in a hole next to seeds or seedlings. Seedlings used in the test were grown in wooden boxes that were filled with pasteurized mineral soil. In the second experiment, seeds and seedlings of different ages (1, 2, 3, or 4 weeks old) were inoculated with 1000 or 5000 eggs of *M. graminicola* per pot (500 cc soil) by placing the inoculum along with seeds or seedlings in the same hole.

*Inoculation Methods*

Different methods of inoculations to assess host resistance have been reported (Starr et al., 2002), but their relative efficiency on the infection and reproduction of *M. graminicola* was not fully known. These different inoculation methods utilizing seeds and seedlings of rice and with two levels of initial inoculum (1000 and 5000 eggs/pot) were tested in a factorial (split-plot randomized) design with 5 replications. The 3 different methods of inoculation (selected based on practical use) evaluated were: mixing the eggs inoculum with additional layer of soil, placing inoculum on top of seeds and covering with soil, and placing inoculum in holes made alongside the seeds or seedlings.

*Relationship of infection severity (RGS) & nematode reproduction on roots (RF)*

This experiment was conducted to understand the relationship between RGS rating and calculated RF for evaluating rice and wheat for resistance to *M. graminicola*. The most common 10 rice and wheat varieties grown in Nepal were screened against an isolate (B 3) of *M. graminicola* from Bangladesh (Padgham,
The test was established in 10-cm clay pots, each was planted with 10 seedlings and inoculated with 5000 (10/cc soil) eggs of *M. graminicola* placed along the seedlings and the pots were incubated in the greenhouse for 60 days. The RGS ratings were regressed against RF value, and root weight; RF values against root weight; and total plant weight, and RF to root weight using Proc GLM SAS (Enterprises, SAS Institute). The same experiment was repeated during the summer months. Thus data sets from the summer and winter tests were combined and RGS ratings and RF values were determined and analyzed.

**Results**

**Incubation Period**

Root galling Severity of (RGS) induced by infections with *M. graminicola* was rather high at both inoculum levels tested (2 and 10 eggs/cc soil) and both incubation periods (45 and 60 days) in rice cv. Mansuli (Table 2.1). However, RGS ratings were higher in the high inoculum density treatment. The calculated RF values were significantly higher at the 60 days incubation treatment as compared to the 45 days incubation treatment at both inoculum levels. However, a significantly higher RF value was obtained at the lower initial inoculum level (2 eggs/cc soil) as compared to the higher inoculum level. The interaction between the two incubation periods and the two-inoculum levels was not significant (P=0.5674).
Inoculum Densities

Initial inoculum level of *M. graminicola* significantly (P=<0.0001) affected RGS ratings that increased with increasing initial inoculum levels from 0.2 to 20 eggs/cc soil and in both the direct-seeded and the transplanted seedling treatments (Figure 2.2). However, the interaction between the inoculum levels and planting materials (seed or seedlings) was not significant, as measured by RGS rating and RF Values. The RGS rating was significantly lower at the 0.2 and 1 eggs/cc soil initial inoculum levels, irrespective of seed or seedling inoculations as compared to those of higher inoculum levels (Figure 2.2).

The level of initial inoculum significantly (P = 0.0471) affected the calculated RF values, and there was no significant difference between RF values of the seed and seedling inoculation treatments. Also, the RF values calculated for the 0.2 and 20 eggs/cc soil inoculum levels were significantly lower than those calculated for the other initial inoculum levels, which were not significantly different from each other (Figure 2.3). Furthermore, the interaction of initial inoculum level and seed or seedling inoculation methods on the nematode reproduction (RF) value was not significant. It was observed that the calculated RF values increased from 0.2 to 2.0 eggs/cc soil initial inoculum level treatments and then decrease at the 10 and 20 eggs/cc levels.

Experimental Unit

Containers (tube and pot ; P = <0.0001), crops (rice and wheat; P = <0.0001), and inoculum levels (0.5, 1, 2, 4, 8 eggs/cc soil; P = < 0.0001) significantly affected the RGS rating in rice caused by *M. graminicola* (Figure 2.4). Similarly, the interaction among inoculum levels (P = <0.0001), container x inoculum level (P =
<0.0001), and crop x container x inoculum level (P = 0.0001) were also significant for RGS rating, whereas crop (P = <0.0001), inoculum level (P<0.001), crop x container (P = 0.0051), crop x inoculum level (P = 0.006) and container x inoculum level (P = 0.0001) were significant for RF values of *M. graminicola* in rice and wheat. Both rice and wheat exhibited higher RGS ratings in pots than those developed in tubes (Figure 2.5). However, significantly higher RF was calculated for the rice and wheat treatments established in tubes than those in pots (Figure 2.5). Also, the RF values calculated for the rice was higher than those for wheat in both containers and at all initial inoculum levels, suggesting that these factors should be considered together when studying the infection and reproduction of this nematode in rice and wheat.

The symptoms produced on wheat and rice roots as a result of infection by *M. graminicola* were also different. Inoculation of rice at seeding with this nematode generally resulted in infections to the main root tips, thereby resulting in stubby root and the development of ‘hook- like’ galls (Figure 2.7B), but this was not common in wheat (Figure 2.7A). The symptoms developed on inoculated rice seedling were on secondary roots and such roots continued growing, thus resulting in less apparent damage than when seeds were inoculated at planting (Figure 2.8).

*Age of Seedling*

No significant difference in RGS ratings was observed on inoculated seedlings of different ages as compared to these of inoculated seeds at planting (Figure 2.9). RF values determined for inoculated seeds and 1 week-old seedlings were not significantly different from each other, but were significantly lower than those developed on older seedlings (Figure 2.10). Higher RGS ratings were observed on plants inoculated with 10 eggs/cc soil as compared to those inoculated with 2 eggs/cc
soil density (P = 0.0001) and the interaction between seedling age x inoculum level (P = 0.0230) was also significant. Inoculation of seeds at planting resulted in severe infections and the development of stubby roots with higher numbers of “hook-like” galls (Figure 2.7 B) as opposed to inoculation of seedlings where gall formation was more prevalent on secondary roots (Figure 2.8).

Significant effects of seedling age at inoculation (P = <0.0001), initial inoculum level (P = <0.00010) and age x inoculum level (P = 0.0030) were also observed on the reproduction of *M. graminicola* (Figure 2.10). The reproduction of this nematode in treatments inoculated with 10 eggs/cc soil produced lower RF values as compared to those of treatments inoculated with 2 eggs/cc soil, irrespective of plant age. However, lower variability in RF values was observed at the 10 eggs/cc soil initial inoculum level as compared to 2 eggs/cc soil (Figure 2.10).

*Inoculation Method*

Lower RF values and RGS ratings were observed in the treatment where the inoculum was mixed with the soil before planting as compared to those developed on seeds or seedlings inoculated by placing the eggs suspension in a close-by holes or spread on the soil surface and covered with a thin layer of soil. The latter two methods of inoculation gave the same results (Table 2.2).

Seedling age (P = 0.0063), seedlings x inoculation method ((P = 0.0074), and inoculum level x seedlings/seeds x inoculation method (P = <0.0001) were significant on the observed RGS ratings, but not the inoculum levels (0.2 or 10). RGS ratings were higher on inoculated seeds as compared to inoculated seedlings. Similarly, RF values of inoculated seedlings or seeds (P = 0.0001), inoculum levels (P = <0.0001) and inoculation methods (P=0.0002), seedling x inoculum level (P=0.0219) and
inoculum level x inoculation method x seedling (P = 0.0005) were significant, but not their interactions. The RF at 5000 eggs/pot initial inoculum and inoculation of seedlings were significantly higher as compared to those of the 20 eggs/cc soil and the seed inoculation treatments.

Relationship of infection severity (RGS) and reproduction (RF) of *M. graminicola*

The preliminary screening of the most commonly grown rice and wheat varieties to *M. graminicola* isolate from Bangladesh indicated that all the tested rice and wheat varieties from Nepal were highly susceptible to this nematode. In rice, RGS rating was significantly correlated (P = 0.0127) with root weight in one experiment, but not in a repeat experiment. However, the RF values were significantly correlated with root weight in both experiments. Also, no significant correlation was found between severity of root infections (RGS ratings) and nematode reproduction (RF values) in both experiments (Figure 2.11). In wheat, a significant correlation between RGS ratings and root weight was not observed, but a significant correlation of RGS ratings and RF values were observed (Figure 2.12). A significantly higher root weight (P=0.0045) is observed in rice and wheat grown in the experiment conducted in the summer than that of the experiment conducted during winter.
Figure 2.2. Influence of initial inoculum density of *M. graminicola* on root galling severity developed on rice cv. Masuli planting seeds or 4 weeks old seedlings. The means in bars with the same letter are not significantly different in each group by LSD (P=0.05) level.

Figure 2.3. Influence of initial inoculum density of *M. graminicola* on reproductive factor values in rice cv. Mansuli established by planting rice seeds or 4-weeks old seedlings. The means in bars with the same letter are not significantly different in each group by LSD (P=0.05) level.
Figure 2.4. Effect of the planting container (small-plastic tube vs 10-cm clay pot) and initial inoculum density (0.5, 1, 2, 4 or 8 eggs/cc soil) on root galling severity produced by *M. graminicola* on rice cv. Mansuli and wheat cv. Brikuti maintained in greenhouse. The means in bars with the same letter in each group are not significantly different at p=0.05 level by LSD.

Figure 2.5. Effect of the planting container (small plastic tube; 150 cc vs 10-cm clay pot; 500 cc) on root galling severity and reproductive factor of *M. graminicola* in rice cv. Mansuli and wheat cv. Brikuti maintained in greenhouse. The means in bars with the different letters in each group are significantly different by LSD (P=0.05 level).
Figure 2.6. Effect of planting container (small plastic tube vs 10-cm clay pot) and initial inoculum density (0.4, 1, 2, 4, or 8 eggs/cc) on reproductive factors of *M. graminicola* in rice cv. Mansuli (A) and wheat cv. Brikuti (B) maintained in greenhouse. The means (bars) with the same letter are not significantly different at P=0.05 level by LSD analysis within each group.
Figure 2.7. Symptom of infection inoculated at seeding by *M. graminicola* on wheat (A) and rice (B) roots.
Figure 2.8. Symptoms on the rice roots inoculated with *M. graminicola* at transplanting.
Figure 2.9. Root galling severity caused by *M. graminicola* on rice cv. Mansuli by inoculating seeds or 1, 2, 3 and 4 weeks-old seedlings with 1000 or 5000 eggs of *M. graminicola*/pot under greenhouse conditions.

Figure 2.10. Reproductive factors (RF) of *M. graminicola* in rice cv. Mansuli as affected by inoculating seeds or 1, 2, 3, and 4 weeks-old seedlings with 1000 and 5000 eggs/pot under greenhouse condition. Data points with the same letter in each line are not significantly different at P=0.05 level by LSD.
Figure 2.11. Correlation of RF values and RGS ratings resulting from infection by *M. graminicola* to root weight (A) and the correlation between RF values and RGS (B) in rice cv. Mansuli in the greenhouse.
Figure 2.12. Correlation of RF values and RGS resulting from infections by Meloidogyne graminicola to root weight (A) and the correlation between RF values and RGS (B) in wheat cv. Brikuti in the greenhouse.
Table 2.1: Effect of incubation time and initial inoculum densities on root-galling severity and reproductive factor *Meloidogyne graminicola* on rice cv. Mansuli.

<table>
<thead>
<tr>
<th>Days after Inoculation</th>
<th>Level of Inoculum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total root Weight&lt;sup&gt;b&lt;/sup&gt;</th>
<th>RGS Ratings</th>
<th>RF values&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>2</td>
<td>6.8 c</td>
<td>6.8ab</td>
<td>366.6 b</td>
</tr>
<tr>
<td>45</td>
<td>10</td>
<td>8.6 a</td>
<td>7.3 a</td>
<td>46.1 d</td>
</tr>
<tr>
<td>60</td>
<td>2</td>
<td>5.8 bc</td>
<td>6.2 b</td>
<td>493.4 a</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>7.4 ab</td>
<td>7.9 a</td>
<td>115.4 c</td>
</tr>
</tbody>
</table>

The means with the same letter are not significantly different by LSD test (P=0.05 level)

<sup>a</sup> Number of eggs per cc soil.

<sup>b</sup> in grams.

<sup>c</sup> RF = Final eggs/initial inoculum.
Table 2.2: Effect of planting materials (seed vs seedling) and inoculum density, and methods on the multiplication of *M. graminicola* on rice cv Mansuli.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>RGS ratings&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RF values&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor A: Inoculum levels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 eggs/cc soil</td>
<td>6.4 a</td>
<td>138.5 b</td>
</tr>
<tr>
<td>10 eggs/cc soil</td>
<td>6.3 a</td>
<td>220.1 a</td>
</tr>
<tr>
<td><strong>Factors B: Seed vs seedling</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seeds</td>
<td>6.1 b</td>
<td>171.6 a</td>
</tr>
<tr>
<td>Seedlings</td>
<td>6.6 a</td>
<td>187.8 a</td>
</tr>
<tr>
<td><strong>Factor C: Inoculation methods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed with soil</td>
<td>6.7 a</td>
<td>186.4 a</td>
</tr>
<tr>
<td>In a hole</td>
<td>5.8 b</td>
<td>158.9 b</td>
</tr>
<tr>
<td>Along seeds/seedlings</td>
<td>6.4 a</td>
<td>194.0 a</td>
</tr>
</tbody>
</table>

In column, means with the same letter are not significantly different within each factors by LSD test (P = 0.05 level).

<sup>a</sup> RGS rating where 1 = no galls (healthy roots) and 9 = >76% roots galled.

<sup>b</sup> RF = Final eggs/initial inoculum.
Discussion

Infection and reproduction of plant-parasitic nematodes depend on the particular host plant (species, cultivar, age at inoculation, nutrition and others), the specific nematode (species, life stage, initial inoculum level, and inoculation method) and environmental conditions (mainly temperature, soil moisture and soil types) (Hussey and Janssen, 2002). The interactive effect of these factors can significantly impact infection severity, crop damage and reproduction of the plant parasitizing nematode. The effects of such interacting factors are often found to be plant species-nematode species and/or race specific. Information on such specificity is already known and available for a number of Meloidogyne species and their hosts. However, such applicable information to *M. graminicola* and rice or wheat is either not available or very limited.

Length and condition of the incubation period is important in assessing nematode damage and its reproduction, thus it is essential in differentiating the effect of tested germplasm and various treatments. It has been suggested that inoculated plants with root-knot nematodes might be evaluated at 40-45 days after inoculation, if incubation temperature is between 25-30 C (Hussey and Janssen, 2002). However, the majority of the studies on root-knot nematodes and various host crops have included an incubation period of 30 days. Previously reported resulted on *M. graminicola* in rice have utilized incubation periods varying from 35 to 90 days (Roy, 1977; Swain and Prasad, 1991; Rao and Israel, 1971; Soriano et al., 1999; Prasad et al., 1986, Yik and Birchfield, 1979). Higher RF values were observed in this study after an incubation period of 60 days as compared to 45 days. The latter likely is due to the maturation of larger number of females after 45 days of inoculation, thus producing more eggs and juveniles with longer incubation time.
Initial inoculum levels are known to affect infection severity and reproduction of many plant-parasitic nematodes on various crops, thereby impacting the observed RGS ratings and final reproduction of the nematode. Greater RF values of *M. graminicola* in rice were observed in this study at a medium inoculation level of 1000 eggs/pot (2.0 eggs/cc soil). Sharma-Poudyal et al. (2005) reported that the optimum build-up of *M. graminicola* was found to be at an initial inoculum density of 5 J2/g soil. The difference in the results reported by the two studies might be due to difference in container (size or materials) and amount and type of soil used. Sharma-Poudyal used large plastic pots containing 5 kg soil, whereas smaller clay pots (10-cm diameter) containing 500 grams of soil were used in this study. Fassuliotis (1985) stated that among the characteristics of successful screening protocol is the effectiveness and optimization of inoculum needed to differentiate the differences among host genotype. The inoculum density selected should not cause extreme injury that mask the identification of potentially useful genetic materials. In addition, such a protocol should also be simple, easy and with high and consistent infection values and no or low escapes. The RF values of *M. graminicola* either in rice or wheat increased from low initial inoculum density (0.25 egg/cc soil) to an intermediate density, (4 egg/pot and 2 egg/tube), but then decreased with increasing initial inoculum densities of upto 25 eggs/cc soil. Maximum reproduction rate of *M. incognita* race 1 in spinach was at of 0.25 egg/cc soil (Di vito et al., 2004), whereas that of *M. javanica* in potato occurred at 4 eggs and larvae/cc soil (Volvas et al., 2005). The reproduction of *M. hapla* decreased exponentially with increasing the initial inoculum density from 0.1 to 20 egg/cc soil on three medicinal plants (Parker et al., 2005). Decrease in the RF values at higher initial inoculum levels may be due to destruction of root systems by high nematode population as a result of competition for nutrition and space among the developing nematodes within the root system. Similar observations have been made
by Mohandas and Ramakrishnan (1997), Pandey and Haseeb (1997) and Park et al. (1999), while studying pathogenicity of *Meloidogyne* on several plant species. However, the increase of root-galling severity with increasing initial inoculum densities may be due to availability of infection sites on roots. These observations are similar to those reported for *Meloidogyne* species in other host plants (Divito et al., 2004; Volvas et al., 2005; Paker et al., 2005; Viaene and Abawi, 1996).

Generally, higher reproductive factors (RF) of this nematode were calculated on rice than wheat. This difference may be linked to root biomass or difference in susceptibility of rice and wheat to this nematode. However, Elkins et al. (1979) emphasized that the difference in root length were more important in response to nematode invasion and reproduction. Wheat is known to have longer root length than rice, thus the greater availability of infection sites. However, Somoroo and Hauge (1992) emphasized the greater role of host resistance on the RF values of the nematode than the effect of root length. In this study, higher RF values were observed in rice than in wheat, which may be due to higher root biomass and/or difference in genetic constituent of the rice and wheat plants. Since different plant species and even varieties might have different reaction to the same nematode, higher RF values in rice may be due to the genetic make-up of the rice plant. However, high variability in the RF values on rice and wheat were observed in different experiments suggesting that other factors were also involved in the reproduction of this nematode in addition to host genotype. Higher RF values obtained by inoculating seedling in comparison to inoculating seeds further support the assumption associating greater nematode numbers with higher root biomass. Similar results of lower reproduction of this nematode in wheat as compared to rice have been reported from India (Gaur and Sharma, 1999), Pakistan (Soomro and Hauge, 1992) and Bangladesh (Padgham et al., 2004). However, Pokharel et al. (2005) observed a few isolates of this nematode to
produce significantly higher RGS and RF values in selected wheat varieties as compared to a few rice varieties and reported a significant interaction between crop variety x isolate of *M. graminicola*.

Utilization of smaller plastic containers, especially for screening purpose, is gaining popularity and has been successfully used in investigations involving a number of *Meloidogyne*-host combinations. Results of this study showed that the size of container affected the maximum RF values of *M. graminicola*. The highest RF values were observed at 2 and 4 eggs/cc soil on rice and wheat growing in plastic tubes (150 cc) as compared to the RF values in clay pots (500 cc). The latter suggested that higher numbers of J2 entered and reproduced in roots of plants growing in the small containers. Hussay and Janseen (2002) reported that larger number of juveniles were able to enter host roots in smaller tubes due to proximity of inoculum to roots, resulting in a higher infection rate. Similar result was observed by Jones et al. (2005), as they found higher numbers of J2 and eggs of *M. incognita* race 3 in roots growing in plastic tubes with 90, 250, 500 and 750 cm3 soil capacity infested with 1000 eggs/tube, but not in those with 1000 cm3 soil capacity. The practical use of small tubes and the required lower inoculum saves space and is cost-effective in screening crop germplasms.

Previous research on the biology and screening for resistance to *M. graminicola* in rice has used germinated seeds (Roy, 1973), un-germinated seeds (Yik and Birchfield, 1979 and Roy, 1977) or seedlings of different ages (Swain and Prasad, 1991; Sharma-Poudyal et al., 2005). Un-germinated seeds are easier to handle and more uniform. Higher reproduction of *M. graminicola* was observed on inoculated seedlings in this study, but higher RGS ratings were observed on inoculated seeds. Higher RGS ratings observed on inoculated seeds at plant maturity may be due to greater and earlier damage to developing root systems. Similar observations were
reported in melons inoculated with *M. incognita* (Ploeg and Phillips, 2001) and on carrots inoculated by *Longidorus africanus* (Huang and Ploeg, 2001), who concluded that nematodes are much more damaging when they are able to attack the developing roots immediately after seed germination. Higher RF values observed on inoculated seedlings may be due to the availability of greater root mass at inoculation time, thus providing more nutrition and infection sites for reproduction of the nematode. Plant age has been shown to influence host efficiency to *M. incognita* in most, but not all the crops tested (Mendoza and Jatala, 1985).

Due to the slow movement of nematode in soil, inoculation methods can have a major impact on infection severity and reproduction of the nematode. Generally, eggs are added into 2-3 depressions in the soil around the stem base of young seedlings (Starr et al., 2002) or close to the planting seed or seedlings. Eggs can also be distributed either by mixing with soil or by mixing with irrigation water during watering. Inoculation methods used in this study significantly affected *M. graminicola* infection and reproduction. A significantly lower RF values were obtained when the inoculum was mixed into soil as compared to placement of the inoculum on the soil surface or in holes around such seeds or seedlings. The latter results were probably due to proximity of eggs placed on soil to developing roots, thus earlier and a higher number of J2 penetrated the roots.

Higher or lower RF values and/or RGS ratings were obtained for similar tests repeated during different months of the year. Generally, higher RF values and higher RGS ratings were observed in experiments conducted during the summer months, despite maintaining a constant greenhouse temperature of 25° C and providing additional hours of light during the winter months. During the winter months, the growth of rice was slow and with poor root development. These results clearly indicated that the growing season had an indirect impact on the reproduction of the
nematode by affecting plant growth. According to Hussey and Janseen (2002), environmental conditions often vary sufficiently in greenhouses between seasons to appreciably affect the results obtained. The inclusion of standard resistant and susceptible genotypes in each screening test will allow normalization of variation resulting from changing environmental conditions. According to Trudgill (1991), host resistance is likely to be sensitive to environmental factors (other than increased temperature), with resistance expression being reduced in environments favorable to pathogen development, and that such resistance factors are more likely to be recessively inherited (Fraser, 1990).

Root galling severity ratings and nematode reproduction are used as parameters for assessing root-knot nematode resistance in crop plants and a classification terminology of host reaction based on these parameters exists (Canto-Saerz, 1985; Dropkin and Nelson, 1960; Robert and Thomson, 1986). The categories of hyper-susceptible and tolerance, as proposed by Dropkin and Nelson, (1960) and Canto-Saenz, (1985) are difficult concept to apply in plant breeding when quantitative ratings are desired. Root galling severity alone is not a satisfactory indicator of root-knot nematode resistance in a number of crops and a preliminary test should be conducted to determine if a strong correlation exists between root-galling severity and nematode reproduction (Hussey and Boerma, 1981).

Generally, there was a lack of correlation between RGS ratings and RF values induced by *M. graminicola* in rice. However, population densities of J2 or eggs of other *Meloidogyne* species have been significantly correlated with root-galling severity or yield of several crops (Birchfiled and Harville, 1984; Hussey and Boerma, 1981; Kinloch, 1982 and Kinloch et al., 1985), but not in soybean (Hussey and Boerma, 1981; Niblack et al., 1986). The latter suggested that root-galling development and nematode reproduction are under the control of independent
genetical factor. Roberts et al. (1998) reported that root-galling is often closely correlated with nematode reproduction, but this is not always the case. Root-galling was reported to be governed by nematode activated chemical release (Trudgill, 1991), but reproduction is governed by host plants (Giebel, 1982). In previous evaluation of rice germplasm against *M. graminicola*, only the root-galling severity (Roy, 1977; Rao and Israel, 1971; Yik and Birchfield, 1979) or only nematode reproduction (Soriano et al., 1999) was considered in characterizing the tested materials. In the absence of a close correlation between RGS ratings and nematode reproduction, selection of both traits was suggested in the development of highly resistant cotton varieties against *Meloidogyne* (Luzzi et al., 1987). To address this problem while working with beans, Mullin et al. (1991) ranked bean germplasm for resistance to root-knot nematode based on calculating a resistance index (RI) that used both root-galling severity and egg mass production, \(RI = \text{root galling severity rating}^2 + \text{egg mass production rating}^2\). However, actual numbers of eggs produced on roots are more reliable than eggs masses estimation in assessing nematode reproduction (Hussey and Janseen, 2002) and estimation of egg masses in *M. graminicola* in rice and wheat is rather difficult as eggs are laid and remain inside the root cortex. Therefore, there is a need to develop an evaluation system (scale) that equally involves RGS ratings and nematode reproduction in the search and development of rice germplasm with resistance to *M. graminicola*. Similar approach will be appropriate in wheat, although employing only one of the factors may not affect the results due to the close correlation found between RGS and RF in wheat.

The overall results of this investigation showed that the inoculation of rice or wheat planted in 10-cm pots (500 cc soil) with 1,000 or 5000 eggs of *M. graminicola* at plating and incubation for 60 days in greenhouse at 25 C was an effective protocol for identifying sources of resistant germplams to *M. graminicola*. The results also
suggested that infection and reproduction of *M. graminicola* in rice and wheat are affected not only by several factors, but also their interaction, which they need to be considered collectively.
Literature Cited


