CHAPTER 3

ISOLATE-BY-CULTIVAR INTERACTION BETWEEN *MELOIDOGYNE GRAMINICOLA* AND SELECTED RICE ANND WHEAT GERMPLASMS•

Abstract

The rice root-knot nematode (*Meloidogyne graminicola*) is widespread and causes yield losses to rice and wheat, important staple grains grown in Nepal and other countries in South and Southeast Asia. Reproduction factor (RF) and root-galling severity (RGS) were measured to test variety by isolate interactions in two experiments in the greenhouse. The first experiment included five isolates of *M. graminicola* (NP 8, NP 12, NP 29, NP 37, and NP 43) and five rice varieties (Mala, Mansuli, Bammorcha, Bonet 73 and Labelle), whereas the second experiment included 3 isolates (NP 29, NP 30 and NP 50) and 11 rice varieties (Ramani, Futuje, Mansala, Bansbareli, Belgudi, Ahe, IR 38, POBRRO10, Bonnet 73 and Labelle). These isolates were obtained from different rice-wheat production fields in Nepal and maintained in the greenhouse. Similarly, the same isolates were also tested with the wheat germplasms (Brikuti, NL 792, BL 1473 and a NY strain) in the first experiment, whereas Annapurna 3, Annapurna 4, BL 1813 and Brikuti were tested in a second experiment. A third experiment was carried out to understand the role of initial inoculum density on the variety by isolate interaction in rice and also wheat. In the latter experiment, two rice (Ahe and Labelle) and two wheat (Brikuti and Annapurna 4) varieties were inoculated with two isolates (NP 29 and NP 50) of *M. graminicola* each at 3 initial inoculum densities. Seeds of rice and wheat were planted in 10-cm clay pots filled with pasteurized soil (30 minutes at 60 C) and inoculated with 5000

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eggs of *M. graminicola* in the first and second experiments, and 1000, 2500 and 5000 in the third. The pots were maintained in a greenhouse at about 25 C, watered daily, and fertilized once a week. After 60 days, the roots were washed free of soil and root-galling severity was rated on a scale of 1 (no visible galls, healthy roots) to 9 (>80% of roots galled). Eggs were then extracted from roots by blending the roots in 1% sodium hypo-chlorite solution for 3.5 minutes and passing the suspension through 100 and 500 mesh screens. Results obtained showed that the Nepalese isolates of *M. graminicola* differed significantly in their reproduction and in RGS ratings on both the rice and the wheat germplasm included in the tests. Also, there was a significant variety by isolate interaction between the isolates of *M. graminicola* and both the rice and the wheat germplasm tested. The RF values of the isolates in rice were generally higher than those in wheat, but with a few exceptions. The time of season when the experiments were conducted affected the nematode infection and reproduction in both rice and wheat. The level of initial inoculum also affected nematode reproduction, which was highest at 1000-2500 and 5000 eggs/pot in rice and wheat, respectively and irrespective of the variety and isolate used. The rice-wheat production system seems vulnerable to the build-up and damage of this nematode.

Introduction

Rice and wheat are important cereals not only in Nepal, but also throughout Asia. Asian populations, which represent half of the world's total, receive about 70 % of their nutrition from rice alone (Datta, 2004). Rice is cultivated mostly in flooded conditions during the rainy season or during summer months with irrigation, whereas wheat is grown in non-flooded soils in the winter season. Both crops can be grown in the same field in succession. Rice-wheat rotation occupies about 30% of the rice and

43% of the wheat cultivated in Nepal. Despite the dissimilarities in rice and wheat cultivation, damage by several insect pest and diseases are common to both crops. Recent studies have shown that *Meloidogyne graminicola* is a major constraint and impacting the productivity of rice-wheat production systems, especially rice productivity in South-East Asian countries (Duxbury, 2004; Personal communication). Other production systems may also be vulnerable to damage by this nematode because of its wide adaptability to flooded and non-flooded conditions, wide host range (attacking cereals, vegetables, and other crops) and its widespread distribution in the region.

The nematode reduced rice yields by upto 30% in farmers' fields in Nepal (Duxbury, 2002), 16-20% in Bangladesh (Padgham et al., 2003) and 30% under greenhouse conditions (Sharma-Poudyal et al., 2005). However, this nematode is also reported to infect wheat in India, Nepal and Bangladesh (Taya and Dabour, 2004; Gaur et al., 1993; Sharma et al., 2001; Pokharel et al., 2005; Munir and Bridge, 2003; Padgham et al., 2004), but the information on the effects of this nematode on yield losses in wheat is limited. In addition, this nematode has also been reported to damage crops other than cereals in recent years (MacGowan and Langdon, 1989).

While management options against this nematode are available, they are not adopted by growers. The use of nematicides is not feasible not only because of health and environmental concerns, but to their affordability and timely availability to resource poor farmers in Asia. Despite the existence of resistant germplasm sources against *M. graminicola* (Bridge et al., 1990; Soriano et al., 1999), the resistance factor has not been incorporated yet into commercial and locally adopted varieties. Plowright et al. (1999) reported that the progeny of a cross between susceptible rice (*O. sativa)* and an accession of *O. glaberrima* (relatively resistant species) were less susceptible to *M. graminicola* than the susceptible parent. However, resistant and adopted varieties

of rice against *M. graminicola* are not available. The use of crop rotation seems to be the only viable option for a sustainable management of this nematode at present.

Isolates of root-knot nematode collected from diverse rice-wheat fields throughout the production regions in Nepal were identified as *M. graminicola* with considerable phenotypic, virulence and genotypic variations (Pokharel et al., 2004a). Variability in the reaction of rice to *M. graminicola* was previously reported by Bridge et al. (1990) and Soriano et al. (1999). Pokharel et al. (2004b) recently reported that commercial rice and wheat varieties from Nepal were susceptible to an isolate of *M. graminicola* collected from Bangladesh. They also observed significant variations in the root-galling severity and the reproduction of *M. graminicola* on the tested varieties. Populations of *M. hapla* from within a single field were found to have different levels of reproductive fitness and to differ in the severity of root-galling induced on selected hosts (Powell, 1957; Mitkowski et al., 2003). Experiments on alfalfa demonstrated that two populations of *M. hapla* had significantly different rates of reproduction (Griffin and McKenry, 1989). It has been suggested that for breeding purpose, the most pathogenic isolates of *M. hapla* should be used for identifying sources of resistance (Van der Beek et al., 1998). This investigation was conducted to elucidate the interaction of representative isolates of *M. graminicola* to selected rice and wheat germplasm. Results obtained from this investigation will also help in deciding which isolate or a mixture of isolates to be used for screening rice and wheat germplasm for resistance to *M. graminicola*.

Materials and Methods

The isolates of *M. graminicola* used in this study were obtained from different rice fields in Nepal (Pokharel et al., 2004). The rice and wheat varieties from Nepal

were provided by the CIMMYT offices in Nepal and Bangladesh, whereas rice germplasm with identified resistance to blast, bacterial blight, insect, physiochemical condition and resistance to rice root-knot nematode were provided by the International Rice Research Institute at Los Banos, Philippine. All experiments were repeated once.

Interactions between isolates of M. graminicola and rice germplasm

In the first experiment, the interaction between 5 isolates of *M. graminicola* (NP 8, NP 12, NP 29, NP 37 and NP 43) and 5 rice germplasms (Mala, Mansuli, Bam-Morcha Bonnet 73 and Labelle) was assessed in the greenhouse. Isolates NP 29, NP 30 and NP 50 were evaluated in the second experiment on 11 rice germplasms (Ramani, Futuje, Mansala, Bansbareli, Belgudi, Ahe, IR 38, POBRRO 10, BH 1442, Bonnet 73 and Labelle). Rice germplasm LA 110 and Labelle were previously reported as resistant and susceptible, respectively, to an isolate of *M. graminicola* from Louisiana (Yik and Brachfield, 1979). The majority of rice and wheat varieties used in the first experiment are grown commercially in Nepal, whereas most of the rice germplasms used in the second experiments have been characterized with potentially resistance factors to other pests and pathogens.

Interactions between isolates of M. graminicola and wheat germplasm

The interactions of the same isolates used in the rice tests were also utilized to assess reaction of 4 additional wheat varieties/lines (NL 792, BL 1473, Brikuti and a NY strain of wheat) in the first experiment and 4 wheat germplasms (Annnapurna 3, Annapurna 4, BL 1813 and Brikuti) in the second experiment. Initial inoculum densities of 5000 eggs per pot were used in these tests on both rice and wheat.

Effect of initial inoculum level on variety by isolate interactions in rice and wheat

In the third experiment, the effects of isolates NP 29 and NP 50 each at 3 initial inoculum densities (2, 5 and 10 eggs/cc soil) on the reaction of rice varieties Ahe and Labelle and the wheat varieties Annapurna 4 and Brikuti were investigated to understand the role of initial inoculum on variety by isolate interaction.

Treatments in the three experiments conducted were arranged in a completely randomized block design with 4 replications. Each treatment replicate consisted of a sterilized 15-cm pot filled with pasteurized (60 C for 30 minutes) mineral soil. Ten rice or 10 wheat seeds were sown per pot, inoculated with eggs of *M. graminicola* and covered with a soil-peat moss mixture. The pots were incubated in a greenhouse maintained at 25 C (Figure 3.1) and received daily watering and fertilized weekly with a solution of complete fertilizers (NPK). After 60 days, plants were uprooted, roots washed free of soil, and root-galling severity (RGS) was determined. A root galling severity rating of 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.

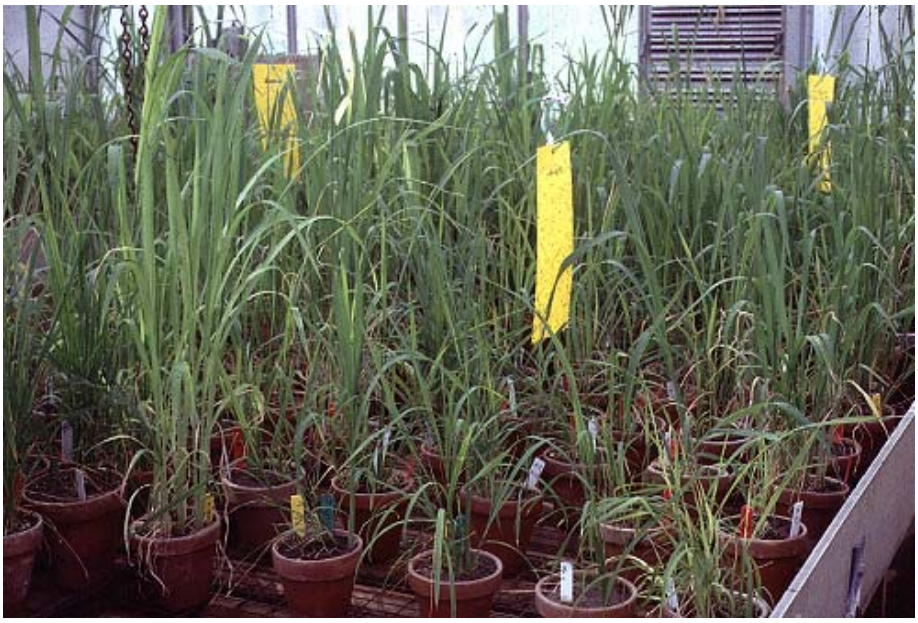

Figure 3.1. Rice germplasm growing in the greenhouse approximately at 60 days after inoculation with *M. graminicola*.

(Mullin et al., 1991). Nematode eggs were then extracted by blending roots for 3 minutes intermittently in 1% sodium hypo-chlorite, the suspension was passed through a # 100 sieve nested on top of a # 500 mesh, washed into a beaker, volume adjusted to 100 ml, dilution series were prepared (if needed) and total eggs by dilution were enumerated under a dissecting microscope. A reproductive factor, [RF =total number of eggs and juveniles extracted divided by 5,000 (the number of eggs used to infest the soil/ pot)], was calculated for each isolate on every variety and germplasm tested. The data were analyzed by ANOVA and mean comparison was done by LSD using Proc GLM (SAS Enterprise guide, SAS, Institute).

Results

Interactions between M. graminicola isolates and rice germplasm:

All the rice varieties included in the first experiment were susceptible to the tested Nepalese isolates of *M. graminicola.* Interestingly, the variety Bonnet 73, previously reported to be resistant to a Louisiana, USA isolate of *M. graminicola,* was found to be susceptible to all the Nepalese isolates tested (3.2 B). A significant effect of variety (P = 0.0102), isolate (P = 0.0007) and variety x isolate interaction (P = 0.0005) was observed on RGS ratings on rice roots. Isolates NP 8, NP 12, NP 29, NP 37 and NP 43 produced the highest RGS ratings on Mala, Labella, Bam-Morcha, Mala and Mansuli, respectively (Table 3.1). The lowest RGS ratings in this experiment were produced by isolate NP 8. The RF of *M. graminicola* isolates was also affected by rice genotype. Isolates NP 8, NP 12, NP 37 and NP 43 produced the highest RF values on Labelle, whereas NP 29 produced the highest RF on Bonnet 73 (Table 3.2). These results suggested a significant effect of variety (P = <0.0001), isolate (P = <0.0001)

and variety x isolate interaction (P = <0.0001) on calculated RF values of *M. graminicola*.

Similar results were obtained in the second experiment with different isolates of *M. graminicola* (NP 29, NP 30, and NP 50) and 11 rice germplasm, including Labelle and Bonnet 73 from the first experiment. Again all rice materials were susceptible to these isolates of *M. graminicola* as suggested by RGS ratings and the calculated RF (Table 3.3 and 3.4). The rice cv. Ramani exhibited the highest RGS ratings to infection by the 3 isolates of *M. graminicola*, whereas the rice cv. Belgudi and Basbareli were among germplasm exhibiting the lowest RGS ratings to infection by the 3 isolates. The other rice germplasm included in the test exhibited RGS ratings that varied greatly among the 3 isolates (Table 3.3). However, there was significant effect of rice germplasm (P = 0.0001), isolate of *M. graminicola* (P = 0.0107) and their interaction (P = 0.0001) on RGS ratings. A significant effect of isolate (P = 0.0032), variety (P = <0.0001) and interactions (P = 0.0002) was also observed on the calculated RF values (Table 3.4). Isolate NP 50 produced highest RF values in most of the varieties. However, RF values of isolates of *M. graminicola* were significantly the highest on Ramani and Labelle, whereas Belgudi and IR 38 had the lowest RF values (Table 3.5).

Interactions between isolates of M. graminicola and wheat germplasm

The four-wheat germplasm included in the first test were susceptible (Figure 3.2 A) to the 5 isolates of *M. graminicola* (Tables 3.5 and 3.6). However, there was a significant effect of wheat germplasm (P = 0.0004), nematode isolate (P = 0.0001) and their interaction (P = 0.00034) on RGS ratings. Isolate NP 8 appeared as the most aggressive, inducing the highest root-galling severity ratings in the 4-wheat germplasm

tested. Similarly, the calculated reproductive factor (RF) values also suggested a significant effect of variety (P = <0.0001), isolate (P=0.00048) and variety x isolate interaction (P=0.0001). Isolates NP 8, NP 12, NP 29, NP 37 and NP 43 produced the highest RF values on NL 792, BL 1473, Brikuti, NY line, and NL 792, respectively. The wheat varieties Brikuti, NL 792, BL 1473 and NY line exhibited the maximum RGS ratings upon inoculation by NP 29, NP 8, NP 12 and NP 29, respectively (Table 3.5).

In addition, all 4 wheat germplasms tested were also susceptible to the isolates of *M. graminicola* tested in the second experiment (Tables 3.7 and 3.8). The effect of isolate (P = 0.01070), germplasm (P = 0.00001) and their interaction (P = 0.0001) on RGS ratings were significant on RGS ratings. Significantly different RGS ratings were caused by different isolates in different wheat varieties. Isolates NP 30 and NP 29 produced the highest RGS ratings on Brikuti and BL 1617, respectively whereas NP 50 produced the highest RGS rating on both Annnapurna 3 and Annapurna 4 (Table 3.7). Only wheat germplasm (P= <0.0001) and interaction between germplasm and isolate (P = 0.0017) were of significant effect on RF values of *M. graminicola.* Isolate NP 30 produced significantly higher RF values on Annapurna 4 and BL 1817, whereas both isolates NP 29 and NP 50 produced the highest RF values on BL 1817 (Table 3. 8).

Effect of initial inoculum density on variety by isolate interactions (rice)

In rice, initial inoculum density significantly (P=0.0005) affected root galling severity, as they increased with increasing inoculum densities from 2 to 10 eggs of *M. graminicola*/pot (Table 3.9). There was a significant affect of inoculum density (P = <0.0001), variety (P = <0.001), density x isolate (P = 0.0019) and variety x density

(P=0.0308) on RGS ratings, but not the isolate or isolate x variety interactions. The average RF values at 5 eggs/cc density was significantly higher than that observed at 10 eggs/pot density, but not at a density of 2 eggs/cc soil (Table 3.10). Inoculum density (P = <0.0001), variety (P = <0.0001), variety x isolate (P = 0.00019) and variety x inoculum density (P= 0.0308) significantly affected RF values (Table 3.10).

Effect of initial inoculum level on variety by isolate interaction (wheat)

In wheat, root-galling severity was significantly affected by initial inoculum density (P = 0.0495) and *M. graminicola* isolates (P = 0.0265). There were significant interactions between inoculum density x isolate (P = 0.0001) and inoculum density x variety x isolate (P = 0.001) on root galling severity ratings. The highest RGS ratings were obtained at the 5000 eggs/pot inoculum densities and were significantly different from those observed at 1000 eggs/pot density (Table 3.11). Similarly, a significant effect of initial inoculum density (P = <0.0001), variety (P = <0.0001), isolate (P = 0.0005), variety x inoculum densities (P = <0.0001), and variety x inoculum densities x isolate (P = <0.0001) on RF was also observed (Table 3.12). The highest RF of the isolates was generally observed at 2500 egg/pot, except that of isolate NP 50 on Annapurna 4 (Table 3.12)

Table 3.1: Root-galling severity ratings caused by five isolates of *M. graminicola* in selected rice varieties (experiment 1).

	Rice varieties					
Isolates Code	Mala	Mansuli	Bam-morcha	Bonnet 73	Labelle	Mean
NP 8	8.3 a A	6.0 b B	2.3 c C	6.3 a B	6.0 b B	5.8 ab
NP 12	5.5 c B	2.5 d D	5.3 b B	4.0 c C	7.8 a A	5.1 b
NP29	6.7 bc AB	5.0 c B	7.3 a A	5.0 b B	6.0 b AB	6.0 a
NP37	6.5 bc A	6.3 b A	5.7 b B	5.5 ab B	5.5 b B	5.9 ab
NP43	7.3 b B	8.0 a A	5.3 b C	5.3 ab C	6.5 b BC	6.5 a
Mean	6.8 A	5.6 AB	5.2 B	5.2 B	5.8 AB	5.9
Contrast:						
Isolate (P = 0.007)						
Variety (P = 0.0102)						
Interaction (P = 0.005)						

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$)

Table 3.2: Reproductive factors produced by selected isolates of *M. graminicola* in selected rice varieties (experiment 1)

	Rice varieties					
Isolates Code	Mala	Mansuli	Bam-morcha	Bonet 73	Labelle	Mean
NP 8	6.0b C	5.8ab C	4.0a C	*19.8a B*	45.4b A	15.8 a
NP 12	9.8b B	3.0b C	9.3a B	4.0b C	14.4c A	8.1 c
NP29	8.5b A	9.3ab A	4.0a B	9.5ab A	7.0d AB	7.6 c
NP37	*19.5a B*	14.3aBC	*8.3a C*	4.9b D	*87.5a A*	26.9 a
NP43	17.8a C	*15.0aBC*	4.8a C	10.8ab BC	56.7ab A	21.0 b
Mean	12.4 B	9.5 BC	6.1 C	9.8 BC	42.2 A	*15.9*

Contrast:

Isolate (P = <0.00001)

variety (P = <0.00001)

variety x isolate (P = <0.00001)

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

Table 3.3: Root-galling severity ratings caused by three isolates of *M. graminicola* in selected rice varieties (experiment 2).

	Isolates						
Varieties	NP 30		NP 29		NP 50		Average
Ramani	8.5	a A	8.8	d A	7.8	d A	8.4 a
Futuje	7.5	ab B	7.5	cd B	3.8	b A	6.3 a
Mansala	7.3	ab B	7.8	cd B	4.5	c A	6.5 a
Bansbareli	2.8	d B	4.5	ab A	2.5	a B	3.3 e
Belgudi	3.5	cd AB	4.0	a A	2.0	a B	3.2 e
S. Masino	7.8	ab A	4.0	a B	4.3	c B	5.4 abc
IR 38	3.8	c AB	5.5	b A	2.5	a B	3.9 cd
POBRRO 10	4.5	de A	4.5	ab A	2.5	a B	3.8 cd
BH 1442	4.8	de B	6.8	b A	3.5	bc B	5.0 abc
Bonnet 73	7.8	ab A	6.3	bc A	4.5	c B	6.2 a
Labelle	6.8	bc B	8.5	d A	8.5	d A	7.9 a
Average	5.9	AB	6.2	A	4.2	B	5.4

Contrast:

Isolate (P = <0.0001)

Variety (P = <0.0001)

Variety x isolate (P = 0.0002)

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

Table 3.4: Reproductive factors of selected isolates of *M. graminicola* in selected rice varieties (experiment 2).

Varieties	Isolates			
	NP 30	NP 29	NP 50	Average
Ramani	267.0a A	331.0 a A	428.0 a A	3342.0 a
Futuje	160.0ab A	129.0ab A	176.0ab A	155.0 b
Mansala	172.0ab C	284.0a B	409.0 a A	288.3 a
Bansbareli	109.8ab A	29.5 cd B	160.8 b A	100.0 c
Belgudi	40.0 cd A	10.8 d B	35.5 d A	28.8 d
S. Masino	222.0ab A	109.0 b A	35.0 d A	122.0 c
IR 38	24.0 d B	35.0 bc B	94.0 bc A	51.0 c
POBRRO 10	74.0 cd A	49.0 bc A	71.0 c A	64.7 c
BH 1442	21.0 d B	31.0 c B	104.0 b A	52.0 c
Bonet 73	10.5 e B	165.0ab A	620.0 a A	265.2 a
Labelle	228.0ab A	263.0a A	226.0 a A	239.0 a
Average	120.8 B	130.6 B	214.5 A	155.3

Contrast:

Isolate (P = 0.0032)

Variety (P = <0.001)

Variety x isolate (P = 0.0002)

The means in a column (small letters and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

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Table 3. 5: Root-galling severity ratings caused by selected isolates of *M. graminicola* in selected wheat varieties (experiment 2).

Isolate	Wheat varieties				
Code	Brikuti	NL 792	BL1473	NYS	Mean
NP 8	5.5a BC	7.8a A	4.5a C	5.8a B	5.9 a
NP 12	5.3a A	4.0ab B	2.5b C	5.0a A	4.9 ab
NP29	2.3b AB	3.3b A	1.8 b B	2.5b AB	3.2 c
NP37	2,5b A	3.3b A	2.5a A	2.0b A	3.2 c
NP43	4.0ab AB	4.0abAB	3.0b B	5.0a A	4.5 b
Average	3.9 A	4.4 A	2.8 B	4.1 A	*4.4*

Contrast:

Isolate (P = 0.0001)

Variety (P =.0004)

Isolate x variety (P = 0.00034)

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

Table 3. 6: Reproductive factors of selected isolates of *M. graminicola* in selected wheat varieties (experiment 2).

Isolates	Wheat varieties				
	Brikuti	NL 792	BL1473	NYS	Mean
NP 8	9.1 ab B	28.1 *a A*	6.2 bc B	6.6 ab B	12.5 a
NP 12	11.4 b BC	6.9 b C	16.0 a *A*	8.0 ab B	10.5 a
NP29	20.4 ab A	11.8 abB	12.5 ab B	10.4 a B	13.7 a
NP37	4.7 b B	4.3 b B	2.6 c C	10.0 a A	5.4 b
NP43	9.5 ab B	22.4 a A	5.2 c B	5.5 b B	10.6 a
Average	11.1 AB	14.7 A	8.5 AB	8.1 AB	*10.6*

Contrast:

Isolate (P = 0.00045)

Variety (P = 0004)

Isolate x variety (P = 0.00034)

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

Table 3.7: Root-galling severity ratings caused by three isolates of *M. graminicola* in selected wheat varieties.

Wheat	Isolates			
Varieties	NP30	NP 29	NP 50	Average
Annapurna 3	6.8 B b	8.5 A a	8.3 A a	7.9 a
Annapurna 4	4.8 B c	3.8 B c	8.3 A a	5.6 c
BL 1813	6.0 B b	8.8 A a	7.3 AB b	7.4 ab
Brikuti	8.3 A a	5.3 B b	6.3 AB c	6.6 b
Average	6.5 B	6.6 B	7.6 A	*6.9*

Contrast:

Isolate (P = 0.0107)

Variety (P = <0.0001)

Variety x isolate (P = 0.0001)

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

Table 3. 8: Reproductive factors of selected isolates of *M. graminicola* in selected wheat varieties (assumed to have narrow genetic base).

	Isolates			
Wheat varieties	NP 30	NP 29	NP 50	Average
Annapurna 3	29 A b	6 B b	23 A b	19.3 c
Annapurna 4	63 A a	15 B b	22 B b	33.3 b
BL 1813	61 B a	351 A a	306 AB a	239.3 a
Brikuti	31 A b	12 A b	48 A b	*30.3 b*
Average	46.0 A	96.0 A	99.8 A	

Contrast:

Isolate (P = 0.0867)

Variety (P = <0.0001)

Isolate x variety (P = 0.0017)

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

Table 3.9: Root-galling severity ratings caused by two isolates of *M. graminicola* in selected rice varieties under different initial inoculum densities.

Inoculum	Ahe		Labelle		
(eggs/cc soil)	NP 29	NP 50	NP 29	NP 50	Average
2	4.0 b B	4.3 b B	6.5 b AB	7.0 b A	6.0 b
5	7.3 a AB	7.5 a AB	8.5 a A	7.7 b B	7.3 ab
10	8.3 A A	8.5 aA	8.4 a A	8.5 a A	8.4 a
Average	6.5 B	6.8 B	7.8 A	7.7 A	7.3

Contrast:

Variety (P = 0.0012)

Isolate (P = 0.0125)

Variety x isolate (P = 0.0213)

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$). .

Table 3.10: Reproductive factors of selected isolates of *M. graminicola* in selected rice under different initial inoculum densities.

Inoculum	Ahe			Labelle				
Levels (eggs/cc soil)	NP 29		NP 50		NP 29		NP 50		Average	
2	317. a	AB	174 b	C	205 b	BC	618 b	A	328	ab
5	321 a	C	508 a	BC	743 a	AB	1415 a	A	747	a
10	36 b	B	109 b	AB	214 b	A	226 c	AB	146	b
Average	224.6	C	263.7	C	387.3	B	753.0	A	*407*	

Contrast:

Level (P = <0.0001)

Variety (P = <0.0001)

Variety x isolate (P = 0.0019)

Variety x level (P = 0.0308)

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

Table 3.11: Root-galling severity ratings caused by three isolates of *M. graminicola* in selected wheat varieties under different initial inoculum densities.

	Wheat varieties				
	Brikuti		Annapurna 4		Average
Density	NP29	NP 50	NP 29	NP 50	
1000	3.3 b AB	3.0 b B	3.8 b A	3.5 b B	3.4 c
2500	5.5 a A	4.5 ab B	5.5 a A	5.3 a A	5.2 b
5000	5.3 a C	6.3 a B	5.3 a C	8.3 a A	6.3 a
Average	4.7 B	4.6 B	4.9 B	5.7 A	

Contrast:

Level (P = 0.0495)

Isolate (P = 0.0265)

Density x Isolate (P = 0.0246)

Level x variety x isolate (P =.0001)

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

Table 3.12: Reproductive factors of selected isolates of *M. graminicola* in selected wheat varieties under different initial inoculum densities.

	Wheat varieties				
	Brikuti		Annapurna 4		Average
Levels	NP 29	NP 50	NP 29	NP 50	Average
1000	12.8 b B	4.8 c C	19.0 b A	13.0 b B	12.4 b
2500	35.3 a B	33.0 a B	40.0 a A	13.0 b C	30.3 a
5000	4.8 c B	5.5 b B	15.0 b A	22.0 a A	11.8 b
Average	17.6 AB	14.4 B	24.7 A	16.0 AB	*18.3*
Contrast:					
Level (P = <0.0001)					
Variety (P = <0.0001)					
Isolate (P = 0.0005)					
Variety x level (P = < 0.0001)					
Level x Isolate (P = <0.0001)					
Variety x level x Isolate (P = <0.0001)					

The means in a column (small letters) and rows (capital letters) followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$).

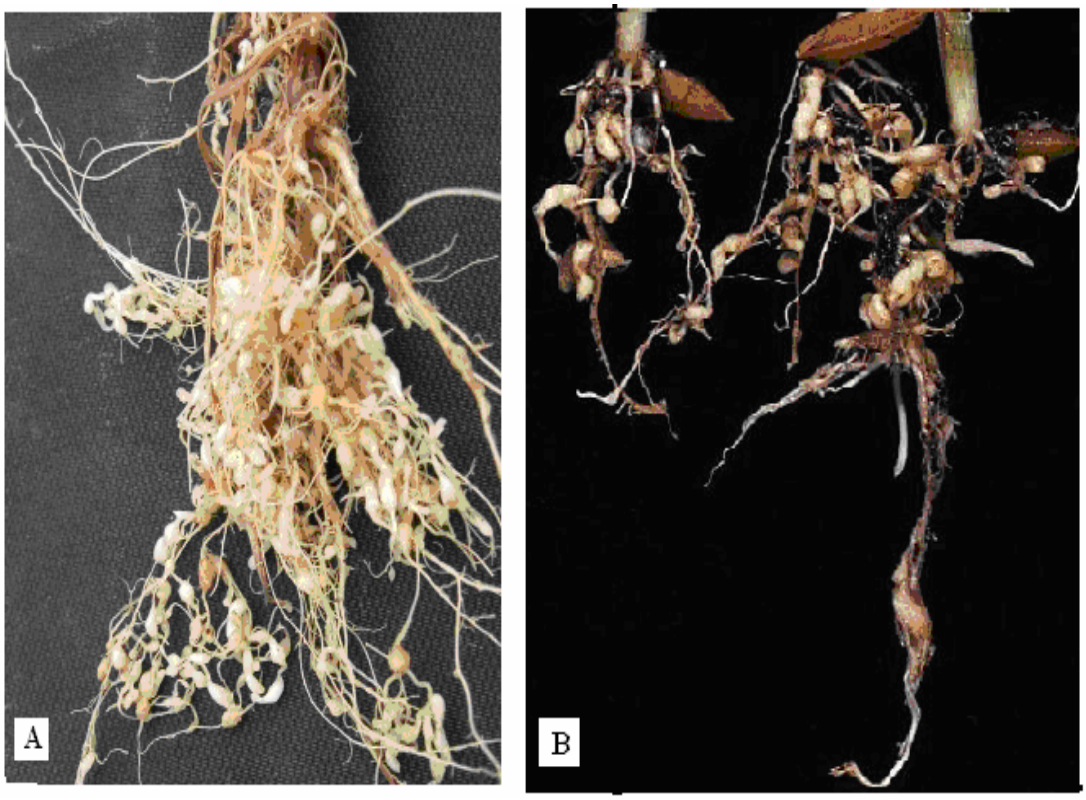

Figure 3.2. Symptoms developed on wheat (A) and on rice (B) inoculated with *M. graminicola* in pots maintained in the greenhouse.

Discussion

The rice varieties Bonnet 73 and LA 110 were previously reported to be resistance to an isolate of *M. graminicola* from Louisiana, USA (Yik and Brichfield, 1979). However, Bonnet 73 and LA 110 were found to be susceptible to all the Nepalese isolates in this study. The latter results may be due to variability in virulence of the isolates used or to greenhouse conditions provided during the tests. Moreover, Yik and Brichfield (1979) based their host reaction determination only on RGS ratings, whereas the present study used both RGS ratings and RF values. In rice, the variety Belgudi exhibited a less susceptible reaction to *M. graminicola* irrespective of the isolate used as compared to the most susceptible varieties Labelle and Ramani. However, there was a significant effect of rice germplasm on RGS and RF caused by the isolates of *M. graminicola*. These results indicated the possible existence of differential resistance genes or alleles in these tested varieties against the Nepalese isolates of *M. graminicola*.

Prior to this investigation, there was no report suggesting the existence of resistant wheat germplasm to this nematode. In addition, only one variety of wheat (Brikuti) was reported to be susceptible to *M. graminicola* in Nepal (Pokharel et al., 2005). Interestingly, the most virulent isolate of *M. graminicola* (NP 37) on rice germplasm was the least virulent on wheat germplasm, whereas isolate NP 50 was found to be the most virulent on wheat in this study. In addition, isolate NP 50 was shown to be among the most virulent on wheat and rice. These results clearly suggest that infection and reproduction of isolates of *M. graminicola* in rice and wheat germplasm are effected by host and pathogen genotypes.

The significant isolate-by-cultivar interaction demonstrated by RGS ratings and reproduction factor (RF) calculated for the Nepalese isolates of *M. graminicola*

suggested the involvement of different genetic factors for virulence in *M. graminicola* and for resistance in rice and wheat. Similar results were reported for *M. hapla* and potato cultivars in Netherlands (Van deer Beek et al., 1998). In addition, Mitkowski and Abawi (2003) observed significant interactions as suggested by the reproductive fitness and root-galling severity between isolates of *M. hapla* and lettuce genotypes. However, Van deer Beek et al. (1998) observed the absence of significant isolate-by-cultivar interactions between *M. chitwoodi* or *M. fallax* and potato cultivars. In the present study, it was surprising to find that all tested rice varieties including the previously reported resistant rice germplasm LA 110 and Bonnet 73, to be susceptible to *M. graminicola* isolates from Nepal. The latter result might be due to either the lack of sources of resistance in these germplasms or to the development of virulence in *M. graminicola* populations.

It has been reported that in the root-knot nematode a/virulence factors can be inherited independently from other characters used for race differentiation (Roberts, 2002). Van deer Beek et al. (1998) studied the reproductive fitness of *M. hapla* on solanaceous species and suggested the possibility of having five *M. hapla* virulence genes. The later was based on the gene-for-gene model as proposed by Flor (1956) and documented for *Globodera rostochiensis* and other cyst nematodes (Janseen et al., 1990). Moreover, presence of a resistant factor in a number of accessions, but not all accessions of wild rice (*O. longistamina and O. glaberrima*), (Soriano et ,1999) further justifies suggesting the evolution of virulence in the isolates of this nematode following the gene for gene model. The suspected genes/factors/alleles in *M. graminicola* seems to have been preserved without a selection pressure from resistance genes, possibly because they have never been introduced before in Nepal. Further studies are warranted to document multiple virulence genes that might exist in populations of *M. graminicola*.

Bonsi (1981) working with isolates of *M. hapla* on lettuce in New York State, observed differences in the reproductive levels between field populations and glasshouse maintained populations of the nematode. He attributed the difference observed in virulence among these populations to the loss of virulence factors as a result of selection in the greenhouse. This is most unlikely to have happened in the present study because precautionary measures were taken by obtaining single female isolates and maintaining the isolates of *M. graminicola* in the greenhouse on a susceptible weed (barnyard grass) and the susceptible rice variety cv. Mansuli. The virulence of isolates was cross-checked randomly in the greenhouse by inoculating the isolates into a rice variety and comparing their reproductive capacity. Moreover, it was further justified by sequence studies in other *Meloidogyne* species where sequence characterization of the SCAR locus showed that alleles between laboratory and field-selected virulent populations were highly similar to each other, but alleles between naturally virulent and (a)virulent populations were distinctly different in *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* (Xu et al., 2003).

Since resistant rice genotypes to *M. graminicola,* including cultivated varieties have been reported (Bridge et al., 1990) and considering the origin of rice and wheat, it is hard to deny the possible existence of gene for gene model (Flor, 1956) in rice and its parasite *M. graminicola*. Rice originated in China and is cultivated extensively, whereas wheat is believed to have originated in Southwestern Asia and is a comparatively new crop to Nepal. Moreover, the extensive use of resistant rice varieties to other pests and pathogens, especially blast and bacterial blight diseases, might have indirectly exerted a selection pressure on this nematode for developing virulent populations.

The virulent populations of *M. graminicola* observed in this study might have developed from avirulent populations either by modified gene action on homologous

chromosome (Baker et al., 1998) or might have been facilitated by acquisition of genetic materials from soil bacteria through horizontal transfer (Scholl et al., 2003), as is the case in other *Meloidogyne* species. This needs further confirmation through molecular investigation. Moreover, dual reproductive behavior of this nematode (both sexual and asexual) might have contributed to higher variability in virulence than in other species having either asexual or sexual reproduction only. It was reported that a pathogen with a mixed reproduction systems pose the greatest risk of breaking down host resistance genes as compared to the pathogens with single type of reproduction (McDonald and Linde, 2002).

It is most likely that virulent populations of *M. graminicola* in Nepal have developed in the absence of resistant host genes, which is further justified by the finding of resistance breaking field populations of other root-knot nematode species elsewhere that have not been previously exposed to resistant cultivars (Netscher, 1977; Robert and Thompason, 1989). Robert and Thompason (1989) suggested that virulence genes are closely related genetically to genes controlling other traits that improve the fitness of the populations. Nevertheless, the possibility of a recent introduction, in the last few decades, of *M. graminicola* from other areas into Nepal could also explain the occurrence of virulent populations of *M. graminicola*. Virulence selection and fitness processes in *Meloidogyne* is complex, as was revealed recently by Castagnone et al. (1996) and Roberts and Mathews (2004).

Variability in root-galling severity caused by isolates of *M. graminicola* in rice and wheat and the higher RF values of isolates of *M. graminicola* in rice as compared to wheat (3 to 10 times higher) might be due to differences in root systems of rice and wheat, resistance genes/alleles in rice and wheat, or the virulence genes or alleles of the Nepalese isolates of *M. graminicola*. Elkins et al. (1979) reported higher reproduction of nematodes on plants with larger and longger roots due to the

availability of greater number of invasion sites. Rice has higher root biomass and higher number of rootlets than wheat. However, Soomro and Hauge (1992) suggested that plant genetic variability is the main factor for higher nematode reproduction in different plants. Similarity in RGS ratings in rice and wheat were observed by Padgham et al. (2004a) and Soomro and Hauge (1992), whereas 2-3 times higher reproduction in rice than wheat was reported by Gaur and Sharma (1999). The latter studies did not measure the RF values.

It was observed in this study that reproduction of isolates of *M. graminicola* was increased as initial inoculum density was increased from 100 to 2,500 eggs/pot irrespective of variety-isolate interactions. However, reproductions of the isolates were generally decreased at initial inoculum density of 5000 eggs/pot. The lower reproduction at higher initial inoculum densities might be due to competition among established females in roots for food and space. However, higher RGS was generally developed at higher initial inoculum densities. The significant interaction of isolate, variety and density of initial inoculum on the infection and reproduction of the nematode on rice and wheat indicated that they are important, but are often neglected.

Results of this investigation suggest that different isolates of *M. graminicola* might be needed for evaluation of rice and wheat for resistance against this nematode, unless a mixture of isolates is used. This finding has important implications for breeders wishing to develop a durable resistance and underscore the importance of evaluating breeder's materials using mixture of populations instead of having single characterized population. Moreover, the rice-wheat production system seems to be a suitable environment for the build-up of this nematode, unless resistant varieties are developed and used. Thus, the use of management practices that reduce the nematode populations in the system should be implemented at present to avoid the increased occurrence and damage of this nematode.

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CHAPTER 4

GREENHOUSE EVALUATION OF RICE AND WHEAT GERMPLSMS FOR RESISTANCE TO *MELODOGYNE GRAMINICOLA*[•]

Abstract

The root-knot nematode (*Meloidogyne graminicola*) is a major constraint to the productivity of rice-wheat systems in South-East Asia. Ninety-six commercial rice varieties and 60 selected rice germplasm with identified desirable traits were evaluated for resistance. Similarly, the reaction to infection by *M. graminicola* of 74 wheat varieties and promising breeding lines was also determined. Ten seeds of rice or wheat were planted per 10-cm clay pots (replicated 4 times), filled with pasteurized soil (30 minutes at 60 C) and inoculated with 10 eggs of *M. graminicola*/ cc soil. The pots were maintained in a greenhouse at a temperature of 25 ±3 C, watered daily, and fertilized once a week. After 60 days, the roots were washed free of soil and root-galling severity was rated on a scale of 1 (no visible galls, healthy roots) to 9 (>76 % of roots galled). Eggs were then extracted from roots by blending the roots in 1% sodium hypo-chlorite solution for 3.5 minutes and passing the suspension through 100 and 500 mesh screens. Eggs on the 500 mesh screen were suspended in water and counted under dissecting microscope. Results obtained suggested that all the commercial varieties and germplasms tested were susceptible to *M. graminicola*. However, both the rice and wheat germplasms differed in their level of susceptibility to *M. graminicola* as evident by the ranges in root-galling severity ratings, reproductive factors observed and the calculated resistant index (RI). Identifying

[•] This chapter will be submitted to Journal of Nematology for publication.

sources of resistance and the development of adopted varieties of rice and wheat to *M. graminicola* is warranted.

Introduction

The productivity of rice and wheat in SE Asia has become stagnant or declining in recent years (Kataki et al, 2001). Results of extensive surveys and research conducted throughout the Gangetic plains have documented that soilborne pathogens and root-health are the major constraints influencing the health and productivity of rice-wheat systems (Duxbury, 2002). The root-knot nematode (*Meloidogyne graminicola* Golden and Brachildfield) is widely distributed and is considered as a serious soilborne pathogen impacting the productivity of the system in SE Asia (Duxbury, 2002; Sharma et al., 2001).

In extensive field tests, solarization of soils infested with root-knot nematode increased rice yield by more than 30% in Nepal (Duxbury, 2002). Also applications of the nematicide carbofuran to infested farmers fields increased rice yield by 16 - 31% (Padgham, 2003). The yield loss caused by this nematode was much higher (31-97%) in greenhouse tests, depending upon the initial inoculum levels (Sharma-Poudyal et al., 2005). Unfortunately the use of nematicides for nematode control and solarization of production fields are not viable options. Moreover, growers are generally not aware of nematode infection and potential yield losses to justify the high cost of such control options. Crop rotation, an effective and sustainable nematode management option, may not be feasible in S.E. Asian countries due to the limited availability of land, seasonal flooding, and the high priority for growers to produce rice.

Thus, the development of nematode-resistant cultivars is the most effective, economical and lasting means for managing nematodes for both large and small-scale

farmers in developing countries. However, only limited breeding efforts have been devoted to developing resistant rice varieties to this nematode (Bridge et al., 1990) by the national research systems in Nepal and Bangladesh and also the International Rice Research Institute (IRRI, Los Banos, Philippines). In addition, very limited information is available on the host-parasite relationship of this nematode in wheat.

Information on the reaction of commercial rice and wheat varieties to this nematode would be useful for designing appropriate crop rotations and for the possible identification of adopted and resistant germplasms for use in the breeding program. Thus, this study was initiated to screen the most commonly grown commercial rice and wheat varieties available in Nepal, Bangladesh and International Rice Research Institute and also promising lines and accessions for their reaction to *M. graminicola.*

Materials and Methods

A total of 156 rice and 74 wheat varieties and selected germplasms were tested against *M. graminicola* in the greenhouse at the NYSAES in Geneva, New York. Twenty-one commercial rice varieties were obtained from International Rice Research Institute (IRRI), Los Banos, Philippines. Also, 38 and 34 commercial rice varieties were obtained from Nepal and Bangladesh, respectively through the International Wheat and Maize Research (CIMMYT) Regional Offices of the respective countries (PPQ526 permit 63098). In addition, 57 rice germplasms having been characterized for resistance to blast, bacterial blight, insect damage, root-knot nematode or adverse physio-chemical properties were also obtained from IRRI. Two resistant rice varieties (Bonet 73, and LA 110) and one susceptible (Labelle) to a Louisiana isolate of *M. graminicola* (Yik and Brachfield, 1979) and also a commercial variety (Cordie) were obtained from the Small Grain Repository Center, Dale Bumpers, Georgia, USA.

Similarly, 24 and 39 wheat varieties and promising breeding materials, respectively were also obtained from the CIMMYT Offices in Nepal and Bangladesh.

All collected rice and wheat varieties and germplasm were evaluated for their reaction to *M. graminicola* in 8 and 4 tests, respectively. Such tests generally included 20 rice or wheat materials and were evaluated using the same protocol and isolates. The isolates of *M. graminicola* used in this study were obtained from rice fields in Nepal and were characterized and maintained in the greenhouse on a susceptible rice variety cv. Mansuli or barnyard grass (E*chinocloa crusgali*) (Pokharel et al., 2004a). The highly virulent isolate of *M. graminicola* from Nepal (NP 50) (Pokharel et al., 2004a) was used in these tests, as a highly virulent isolate can discriminate genotypes with the highest level of resistance (Hussey and Janseen, 2002). All germplasms exhibiting a reproductive factor (RF) of < 10.0 and root-galling severity < 3.0 in the first tests were re-evaluated using 3 different isolates of *M. graminicola* (NP 29, NP 30 and NP 50) at an inoculum density of 10 egg/cc soil (5000 eggs per pot).

Evaluated entries in all tests were arranged in a completely randomized block design with 4 replications. Each replicate of rice or wheat to be evaluated consisted of a sterilized 15-cm pot filled with pasteurized (60 C for 30 minutes) mineral soil. Ten rice or 10 wheat seeds were sown per pot, inoculated with 10 eggs of *M. graminicola*/cc soil and covered with a soil-peat moss mixture. Planted pots were incubated in a greenhouse maintained at 25^{o} C for 60 days and received daily watering and weekly fertilization with a solution of complete fertilizers (10-10-10, NPK).

After 60 days, plants were uprooted, roots washed free of soil and severity of root-galling was determined on a 1-9 scale by estimating proportion of roots galled. A root galling severity rating of 1 = no galls observed (healthy roots), 2 = ≤ 5%, 3 = 6-10%, 4 = 11-18%, 5 = 19-25%, 6 = 26-50%, 7 = 51-65%, 8 = 66-75%, and 9 = 76-100% of root galled (Mullin et al., 1991). Nematode eggs were then extracted by

blending roots for 3 minutes intermittently in 1% sodium hypo-chlorite, the suspension was passed through a # 100 sieve nested on top of a # 500 mesh, washed into a beaker, volume adjusted to 100 ml, dilution series were prepared (if needed) and total eggs by dilution were enumerated under a dissecting microscope. A reproductive factor, [RF = total number of eggs and juveniles extracted divided by 5,000 (the number of eggs used to infest the soil/ pot)], was calculated for each isolate on every variety and germplasm tested. The data was analyzed by ANOVA and mean comparison was done by LSD using Proc GLM (SAS Enterprise guide, SAS, Institute).

Due to lack of correlation between RGS and RF (Pokharel et al., 2004b), both factors were used in determining the reaction of tested rice and wheat germplasm to *M. graminicola*. Thus, Reaction Index (RI) was calculated as was proposed by Mullin et al. (1991), with some modification since egg masses of this root-knot nematode species are deposited inside the roots. In this study, RF values were converted the into a 1-9 scale based on RF values as percentage of that of the susceptible check variety (Labelle in rice and Brikuti in wheat). The percent reproductive factor (PRF) were converted to RF scale as follows: 1= 0, 2 =1-10%, 3=11-20%, 4= 21-30%, 5=31-40%, 6= 41-50%, 7 = 51-60%, 8=61-70% and 9= >70% of the nematode reproduction on the susceptible variety. Since the susceptible checks rice cv. Labelle and wheat cv. Brikuti used in the various experiments exhibited different RGS. The observed RGS rating of the tested germplasm in each test were also converted to percentage as compared to the susceptible check. The calculated % RGS was converted to scale of 1-9. The reaction index of the tested materials was determined following formula suggested by Mullin et al. (1991) RI = RGS rank^2 + RF rank^2. In this scheme, the reaction of a plant to root-knot nematode was classified as: immune (I), RI = 2.0; as highly resistant (HR), RI = < 4.0; as Resistant (R), RI = 4.1-18; as moderately resistant (MR), RI =

18.1-50; as Intermediate (IM), RI = 51-71; as susceptible (S), RI = 72-98; and as Highly susceptible (HS), RI = > 99.

Results

All the commercial rice varieties obtained from IRRI and the National Research Centers (NRC) in Nepal and Bangladesh (a total of 96 varieties) exhibited a susceptible reaction to *M. graminicola* in the greenhouse tests (Figure 4.1 A) and there was considerable variability in the reaction of tested germplasm in repeated tests. However, these diverse rice varieties and germplasms differed greatly in their level of susceptibility to *M. graminicola* within a test, as evident by the variability in RGS and RF values. However, most of the varieties exhibited highly susceptible reaction, as indicated by the calculated RI (Tables 4.1 to 4.5). For example, the RGS values of the varieties developed by IRRI and the NRC in Nepal and Bangladesh varied between 2.0 – 9.0, 4.0 - 9.0, and 3.5 - 9.0, respectively. Similarly, the RF values of the same varieties varied between 4.3 - 20.2, 5.0 - 96.8, and 4.8 - 146.0, respectively. The commercial rice varieties POBRRE 10, IR 5/672 and BARI 27 from IRRI, Nepal and Bangladesh, respectively exhibited the lowest RGS ratings; whereas POBRRE 10, BH 1442 and BARI 24 from IRRI, Nepal and Bangladesh, respectively exhibited the lowest RF values, indicating high variability in RGS values and calculated RF among the tested varieties. In addition, the RGS and RF values for the known susceptible rice cv. Labelle, which was included in all the tests as a check, also varied greatly from test to test. The RGS of Labelle ranged from 4.8 - 9.0, whereas the RF values varied between 14.3 - 121.0. The latter probably was due to effect of the greenhouse environment during the year on the growth of rice and infection and reproduction efficiency of the nematode.

The reaction of 60 rice germplasm with identified desirable characters (resistance to other pests and diseases) was also found to be susceptible to *M. graminicola* (Table 4.4). Higher levels of variability in RGS (1.8 - 9.0) and RF values (3.5 - 187.8) were observed in these germplasms as compared to that observed among the commercial varieties tested. Again, there was considerable difference in the RGS and RF values of the tested rice germplasm within and between the 3 tests conducted (Table 4.4). However, higher variability in RI was observed in germplasms having resistant genes to other pest and diseases. In addition, variable reaction was again observed when the commercial varieties and germplasms with the lowest RGS (<3.0) and lowest RF (<10.0) from the initial tests were re-evaluated (Table 4.5). The RF values ranged from 35.0 – 409.0 whereas the RGS ratings ranged from 2.5 to 7.5. Balamchi, Sulidhan Masino, Baram Kartika and PRBBRO 10 exhibited the lowest RGS rating in both initial and repeated experiments, whereas the remaining re-tested varieties showing lower RGS rating in initial tests exhibited a susceptible to highly susceptible reaction.

All the commercial wheat varieties obtained from the National Research Centers (NRC) in Nepal and Bangladesh (total of 69) as well as the 5 germplasm from USA exhibited a susceptible reaction to *M. graminicola* in greenhouse tests (Figure 4.1 B). However, the commercial wheat varieties differed considerably from each other in their RGS, RF values produced by *M. graminicola* and the calculated RI (Table 4.6 and 4.7). For example, The RGS ratings of the commercial wheat varieties and parental lines obtained from the NRC in Nepal and Bangladesh ranged from 1.9 - 9.0 and 3.5 - 8.5, respectively. Generally, higher RF values for this nematode were observed among the varieties from Nepal (1.3 – 87.0) as compared to the varieties from Bangladesh (5.8 - 65.8). The lowest RGS and RF values were exhibited by the Nepalese germplasm Annapurna and BL 1887, respectively; whereas the Bangladesh

germplasms BAW 972 and BAW 284 exhibited the lowest RGS and RF values, respectively. Unfortunately, the commercial varieties and/or parental lines with the lowest RGS and/or lowest RF values from each test were found to be highly susceptible when re-evaluated in later tests. RGS ranged from 2.5 to 7.8 and RF values ranged from 35.5 - 430.0. The wheat variety Brikuti, used as a susceptible check (Pokharel et al., 2004b) in the different tests also exhibited variable RGS and RF values ranging from 4.8 – 7.8 and 16.8 – 37.0, respectively. This wheat variety was also found with higher RF values in the repeat experiments conducted during summer months as compared to its reaction in the initial test. Rice and wheat varieties were categorized into different reaction groups of susceptibility based on their calculated RI from the observed RGS and RF values (Tables 4.1 – 4.8). The wheat germplasm BL 1887 appeared relatively less susceptible to *M. graminicola* as compared to the rest of the varieties and parental lines tested.

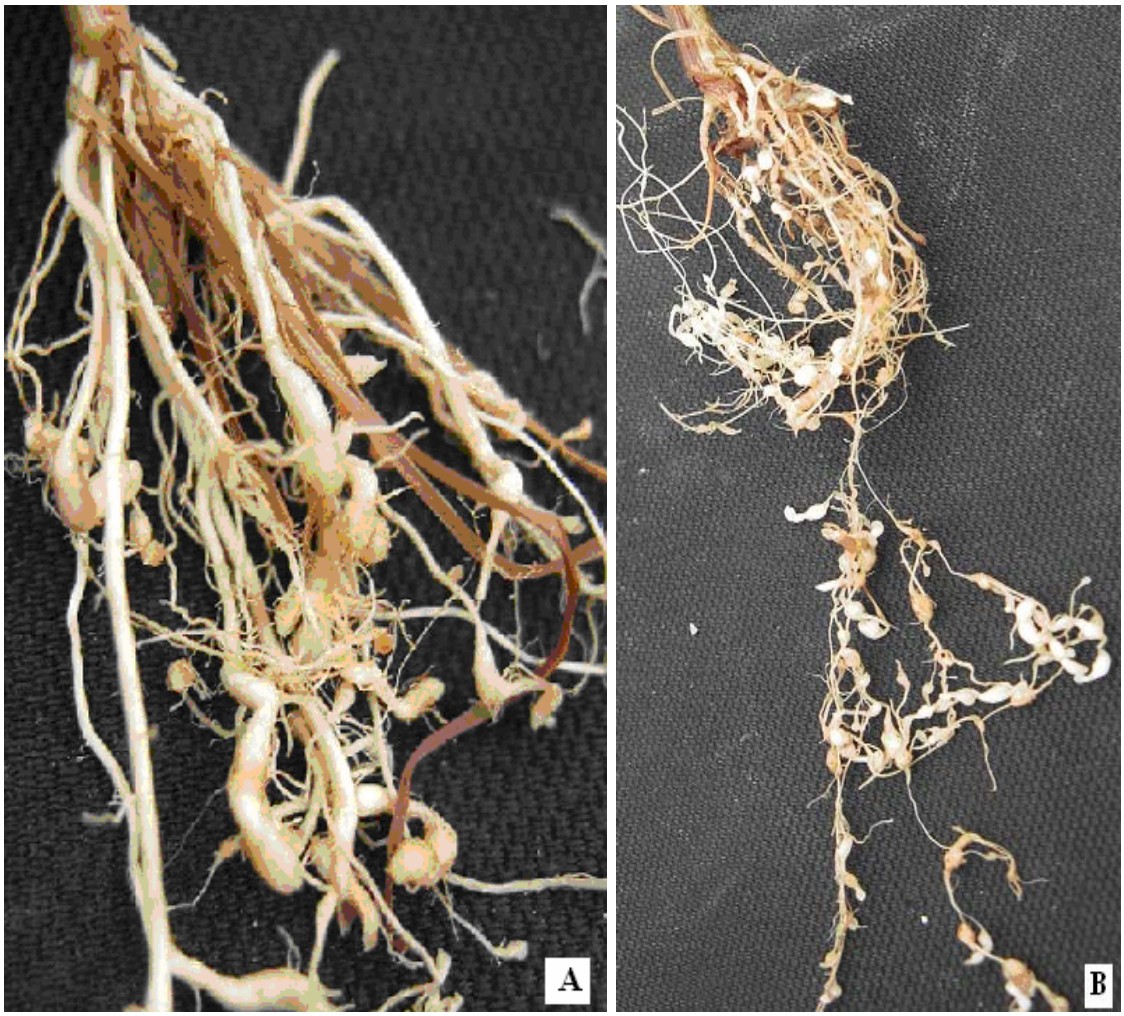

Figure 4.1. Root-knot symptoms in susceptible rice cv. Labelle (A) and on wheat cv. Brikuti (B) varieties following inoculation with *M. graminicola* in greenhouse test.

Table 4.1: Root-galling severity (RGS) ratings and reproductive factors (RF) resulted from inoculation of commercial rice varieties developed by Agricultural Systems in Nepal with *M. graminicola,* isolate NP 50.

	RF			RGS			RI	
Varieties	Actual[a]	%of ck[b]	Rank[c]	Actual[d]	%of ck[e]	Rank[f]	Score[g]	Reaction[h]
	16.3	74.4	9	3.0	50.0	6	117	HS
Chaite-2	7.5	34.2	5	4.5	75.0	8	89	S
Ghaya-2	29.5	134.7	9	4.0	66.7	8	145	HS
NR 1487	15.5	70.8	9	4.8	80.0	9	162	HS
Radha-11	16.0	73.1	9	5.0	83.3	9	162	HS
Radha ?	9.3	42.5	6	5.0	83.3	9	117	HS
Radha-4	6.0	27.4	4	8.0	133.3	9	97	HS
Bindeshowri	13.3	60.7	8	6.7	111.7	9	145	HS
Bp! 3-2	8.0	36.5	5	6.0	100.0	9	106	HS
Chaite-6	7.8	35.6	5	6.4	106.7	9	106	HS
B W. 306 [H]	29.0	132.4	9	6.3	105.0	9	162	HS
Bp! 3-2 [H]	96.8	442.0	9	6.8	113.3	9	162	HS
B H. 1442	5.0	22.8	4	7.3	121.7	9	97	S
NR 601-1-1-5	15.3	69.9	8	7.0	116.7	9	145	HS
B W. 306	13.3	60.7	7	8.5	141.7	9	130	HS
CH 45	9.5	43.4	6	8.4	140.0	9	117	HS
Chaite-4	18.3	83.6	9	8.4	140.0	9	162	HS
Janaki	23.5	107.3	9	8.4	140.0	9	162	HS
NR 1488	7.3	33.3	4	9.1	151.7	9	97	S
NR 601-11-9	13.0	59.4	6	9.3	155.0	9	117	HS
Pusha 834	26.8	122.4	9	9.3	155.0	9	162	HS

(Table 4.1 continued)

Labelle	21.9	100.0	9	9.1	151.7	9	162	HS
Achame Masino	14.1	64.4	8	8.0	133.3	9	145	HS
Anadi	10.4	47.5	6	7.0	116.7	9	117	HS
Bam Morcha	9.0	41.1	6	5.0	83.3	9	117	HS
Kanchi masuli	9.5	43.4	6	8.0	133.3	9	117	HS
Mala	16.9	77.2	9	7.0	116.7	9	162	HS
Malasiya	14.6	66.7	8	7.0	116.7	9	145	HS
Masuli	13.0	59.4	7	8.0	133.3	9	130	HS
Rampur Mansuli	13.0	59.4	7	9.0	150.0	9	130	HS
Seto Mansuli	9.0	41.1	6	7.0	116.7	9	117	HS
Laxmi	8.0	36.5	5	6.0	100.0	9	106	HS
Radha-7	6.0	27.4	4	8.5	141.7	9	97	S
Radha-9	5.3	24.2	4	8.5	141.7	9	97	S
Radha-32	9.0	41.1	6	9.0	150.0	9	117	HS
Makawanpur-1	6.5	29.7	4	6.0	100.0	9	97	S
Radha-12	5.3	24.2	4	6.3	105.0	9	97	S
CH 45 [H]	34.9	159.4	9	5.5	91.7	9	162	HS
Radha-17	6.8	31.1	5	6.5	108.3	9	106	HS
Labelle	22.0	100.0	9	6.0	100.0	9	162	HS

P =0.0012 By LSD tests for RI

[a] Refers to Actual RF {final nematode populations/initial inoculum of 10 eggs/cc soil.

[b] Refers to percentage of RF as compared to that of susceptible check, which was considered 100%.

[c] The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared susceptible check.

(Table 4.1 continued)

[d] Refer to root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled).

[e] Refers to percentage of RGS as compared to that of susceptible check, which was considered 100%.

[f] Refers to rank converted to a scale of 1-9.

[g] Refers to score = RF $rank^2$ + RGS $rank^2$.

[h] Refers to Resistant Index: Immune = < 2.0, Highly resistant =<4.0, Resistant = < 18, Intermediate = <71, susceptible = < 98 and highly susceptible = > 99.

Table 4. 2: Root galling severity (RGS) ratings and reproductive factors (RF) resulted from inoculating commercial rice varieties developed by International Rice Research Institute, Philippines with *M. graminicola,* isolate NP 50.

	RF			RGS			RI	
Varieties	Actual[a]	%of ck[b]	Rank[c]	Actual[d]	%of ck[e]	Rank[f]	Score[g]	Reaction[h]
IR 22 [F]	116.0	79.5	9	7.5	88.2	9	162	HS
IR 32[F]	35.8	24.5	4	3.8	44.7	7	65	IM
IR 5 [F]	78.3	53.6	7	7.8	91.8	9	130	HS
IR 24 [F]	202.0	138.4	9	8.0	94.1	9	162	HS
IR 28 [F]	95.3	65.3	8	8.3	97.6	9	145	HS
IR 8 [F]	69.3	47.5	6	8.5	100.0	9	117	HS
POBRRE 10 [F]	5.0	13.4	3	2.3	27.1	6	45	MR
POBRRE 4 [F]	4.3	2.9	2	4.8	56.5	7	53	IM
IR 36 [F]	9.0	6.2	2	5.5	64.7	8	68	IM
IR 50 [F]	11.8	8.1	2	5.5	64.7	8	68	IM
IR 38 [F]	7.4	14.6	3	2.5	29.4	6	45	MR
IR 58 [F]	23.0	15.8	3	5.0	58.8	7	58	IM
IR 42 [F]	54.0	37.0	5	6.0	70.6	8	89	S
IR 20 [F]	15.0	10.3	3	7.0	82.4	9	90	S
IR 26 [F]	12.0	8.2	2	8.0	94.1	9	85	S
IR 60 [F]	46.0	31.5	5	8.0	94.1	9	106	HS
IR 54 [F]	79.0	54.1	7	8.0	94.1	9	130	HS
IR 46 [F]	10.0	6.8	2	9.0	105.9	9	85	S
IR 64 [F]	146.0	100.0	9	9.0	105.9	9	162	HS
IR 66 [F]	25.5	17.5	3	6.5	76.5	9	90	S
IR 62 [F]	68.0	46.6	6	7.5	88.2	9	117	HS

(Table 4.2 continued)

Labelle	50.3	34.5	5	8.5	100.0	9	106	HS

P = 0.0013 by LSD test

[a] Refers to Actual RF {final nematode populations/initial inoculum of 10 eggs/cc soil.

[b] Refers to percentage of RF as compared to that of susceptible check, which was considered 100%.

[c] The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared susceptible check.

[d] Refer to root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled).

[e] Refers to percentage of RGS as compared to that of susceptible check, which was considered 100%.

[f] Refers to rank converted to a scale of 1-9.

[g] Refers to score = RF $rank^2$ + RGS $rank^2$.

[h] Refers to Resistant Index: Immune = < 2.0, Highly resistant =<4.0, Resistant = < 18, Intermediate = <71, susceptible = < 98 and highly susceptible = > 99.

Table 4.3: Root galling severity (RGS) ratings and reproductive factors (RF) resulted from inoculating commercial rice varieties developed by Agricultural Systems in Bangladesh *M. graminicola* , isolate NP 50.

	RF			RGS			RI	
Varieties	Actual[a]	%of ck[b]	Rank[c]	Actual[d]	% of ck[e]	Rank[f]	Score[g]	Reaction[h]
BARI 14	8.5	54.8	7.0	5.0	55.6	7	98	S
BARI 15	16.8	108.4	9.0	6.3	70.0	8	145	HS
BARI 12	8.5	54.8	7.0	6.8	75.6	9	130	HS
BARI 10	4.8	31.0	5.0	7.0	77.8	9	106	HS
BARI 11	7.0	45.2	6.0	7.0	77.8	9	117	HS
BARI 17	11.3	72.9	9.0	7.3	81.1	9	162	HS
BARI 2	14.5	93.5	9.0	7.5	83.3	9	162	HS
BARI 3	10.8	69.7	8.0	7.5	83.3	9	145	HS
BARI 5	7.0	45.2	6.0	7.8	86.7	9	117	HS
BARI 18	7.5	48.4	6.0	7.8	86.7	9	117	HS
BARI 6	29.0	187.1	9.0	8.0	88.9	9	162	HS
BARI 16	14.0	90.3	9.0	8.0	88.9	9	162	HS
BARI 9	26.0	167.7	9.0	8.3	92.2	9	162	HS
BARI 1	17.8	114.8	9.0	8.5	94.4	9	162	HS
BARI 8	12.8	82.6	9.0	8.8	97.8	9	162	HS
BARI 7	19.0	122.6	9.0	8.8	97.8	9	162	HS
Labelle	15.5	100.0	9.0	9.0	100.0	9	162	HS
BARI 27	37.5	241.9	9.0	3.5	58.3	7	130	HS
BARI 26	11.3	72.9	9.0	4.5	75.0	8	145	HS
BARI 19	46.5	300.0	9.0	4.8	80.0	9	162	HS
BARI 32	15.5	100.0	9.0	5.3	88.3	9	162	HS

(Table 4.3. continued)

BARI 21	67.0	432.3	9.0	5.8	96.7	9	162	HS
BARI 30	13.5	87.1	9.0	5.8	96.7	9	162	HS
BARI 33	9.8	63.2	8.0	5.8	96.7	9	145	HS
BARI 22	145.0	935.5	9.0	6.3	105.0	9	162	HS
BARI 23	60.3	389.0	9.0	6.3	105.0	9	162	HS
BARI 34	16.5	106.5	9.0	6.5	108.3	9	162	HS
BARI 25	108.5	700.0	9.0	6.8	113.3	9	162	HS
BARI 24	2.8	18.1	3.0	7.0	116.7	9	90	S
BARI 31	37.5	241.9	9.0	7.3	121.7	9	162	HS
BARI 29	146.0	941.9	9.0	8.0	133.3	9	162	HS
BARI 28	111.5	719.4	9.0	8.3	138.3	9	162	HS
BARI 37	14.5	93.5	9.0	6.7	111.7	9	162	HS
BARI 38	12.7	81.9	9.0	7.2	120.0	9	162	HS
BARI 39	5.8	37.1	5.0	5.0	83.3	9	106	HS
BARI 40	14.0	90.3	9.0	7.2	120.0	9	162	HS
Labelle	73.3	472.9	9.0	6.0	100.0	9	162	HS

P = 0.0024

[a] Refers to Actual RF {final nematode populations/initial inoculum of 10 eggs/cc soil.

[b] Refers to percentage of RF as compared to that of susceptible check, which was considered 100%.

[c] The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared susceptible check.

[d] Refer to root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled).

[e] Refers to percentage of RGS as compared to that of susceptible check, which was considered 100%.

[f] Refers to rank converted to a scale of 1-9.

[g] Refers to score = RF rank^2 + RGS rank^2.

(Table 4.3 continued)

[h] Refers to Resistant Index: Immune = < 2.0, Highly resistant =<4.0, Resistant = < 18, Intermediate = <71, susceptible = < 98 and highly susceptible = > 99.

Table 4. 4: Root galling severity (RGS) ratings and reproductive factors (RF) resulted from inoculating rice germplasms having resistant genes to other pathogens, insect and physio-chemical characters with *M. graminicola,* isolate NP 50.

	RF			RGS			RI	
Varieties	Actual[a]	%of ck[b]	Rank[c]	Actual[d]	%of ck[e]	Rank[f]	Score[g]	Reaction[h]
Putuje[D]	3.5	24.4	3	3.3	68.8	8	73	S
Ramani[A]	7.8	54.5	7	3.5	72.9	8	113	HS
Lal Anhu[A]	17.0	118.8	9	3.5	72.9	8	145	HS
Jumula-2[D]	10.5	73.4	9	4.3	89.6	9	162	HS
Duram[A E]	7.5	52.4	7	4.8	100.0	9	130	HS
Lamani[B]	16.5	115.3	9	5.0	104.2	9	162	HS
Bageri[C]	16.5	115.3	9	5.0	104.2	9	162	HS
C-5905[A]	10.3	72.0	8	5.3	110.4	9	145	HS
Palpalee[C]	10.5	73.4	8	5.3	110.4	9	145	HS
Mansala[D]	6.3	44.0	6	5.3	110.4	9	117	HS
Tally[D]	25.3	176.9	9	5.3	110.4	9	162	HS
Jalmani[A]	15.3	106.9	9	5.5	114.6	9	162	HS
Garem[AE]	16.3	113.9	9	5.8	120.8	9	162	HS
Jarmani[B]	11.0	76.9	9	6.0	125.0	9	162	HS
Gadur[C]	15.8	110.4	9	6.0	125.0	9	162	HS
Sokan[C]	23.8	166.4	9	6.3	131.3	9	162	HS
Jarneri[B]	17.0	118.8	9	6.5	135.4	9	162	HS
Jinuwa[B]	15.3	106.9	9	6.8	141.7	9	162	HS
Ghure[A]	26.3	183.9	9	6.8	141.7	9	162	HS
Labelle	14.3	100.0	9	4.8	100.0	9	162	HS

(Table 4.4 continued)

Sulidhan Masino[B]	5.3	18.2	3	3.3	41.3	6	45	MR
Thulo Achheme[D]	11.8	40.6	6	3.8	47.5	6	72	S
Basbarelli[A]	64.0	220.6	9	5.0	62.5	7	130	HS
Balumsan[B]	10.5	36.2	5	5.3	66.3	8	89	S
Paheli[D]	35.3	121.7	9	5.5	68.8	8	145	HS
Phulpate	53.8	185.5	9	5.8	72.5	8	145	HS
Ahe (local)[B]	26.3	90.6	9	6.0	75.0	8	145	HS
Tina Sary[B]	18.5	63.7	8	6.8	85.0	9	145	HS
Sano B. Chiya[A]	5.0	17.2	3	7.0	87.5	9	90	S
Belgudi[A]	37.0	127.5	9	7.0	87.5	9	162	HS
Chengul (Bine)[B]	20.5	70.6	8	7.3	91.3	9	145	HS
Amaghaud[A]	27.0	93.1	9	7.7	96.3	9	162	HS
Kamod[B]	32.0	110.3	9	7.8	97.5	9	162	HS
Basmai[B]	23.0	79.3	9	8.0	100.0	9	162	HS
Garue Ghaiya[D]	14.5	50.0	6	8.0	100.0	9	117	HS
R 146[C]	57.5	198.2	9	8.3	103.8	9	162	HS
Suga Pankhe[D]	99.5	343.1	9	8.3	103.8	9	162	HS
Sajani[A]	69.5	239.6	9	8.5	106.3	9	162	HS
White Atte[C]	40.5	139.6	9	8.5	106.3	9	162	HS
Labelle	29.0	100.0	9	8.0	100.0	9	162	HS
Balamachi[A]	6.5	13.0	3	1.8	20.0	5	34	MR
Baram Kartika[A]	5.3	10.6	3	3.5	38.9	6	45	MR
Kanegira[D]	33.0	66.0	7	3.8	42.2	6	85	S
Bengsar[A]	30.5	61.0	8	5.3	58.9	7	113	HS

(Table 4.4 continued)

Suga Pankha[D]	54.5	109.0	9	6.0	66.7	8	145	HS
Thuli Dhan[B]	77.3	154.6	9	6.5	72.2	8	145	HS
Simtharo[D]	105.5	211.0	9	6.5	72.2	8	145	HS
Bhadiya Dhan[B]	187.8	375.6	9	7.5	83.3	9	162	HS
Tulsiphul[B]	118.0	236.0	9	7.5	83.3	9	162	HS
DheradunBasmat [D]	130.3	260.6	9	9.0	100.0	9	162	HS
Tunde[B]	174.3	348.6	9	9.0	100.0	9	162	HS
Balamachi[B]	6.5	13.0	3	1.8	20.0	9	90	HS
Zeena Masino[B]	131.3	262.6	9	7.8	86.7	9	162	HS
Katakana [g]	73.0	146.0	9	8.0	88.9	9	162	HS
Panbira [g]	16.0	32.0	5	8.5	94.4	9	106	HS
H. kalmi [g]	7.5	15.0	3	5.5	61.1	9	90	S
M. Bati [g]	5.8	11.6	3	7.8	86.7	9	90	S
Dular [g]	10.3	20.6	4	7.0	77.8	9	97	S
Deharil [g]	13.3	26.6	4	7.0	77.8	9	97	S
LA 110 [E]	23.5	47.0	6	7.4	82.2	9	117	HS
Bonnet[E]	69.2	138.4	9	6.8	75.6	9	162	HS
Cordie	67.4	134.8	9	8.8	97.8	9	162	HS
Labelle	50.0	100.0	9	9.0	100.0	9	162	HS

P = 0.0014

[A] Resistance to Bacterial Blig.
[B] Resistance to Blas.
[C] Resistance to insec.
[D] Resistance to Physio-chemical.
[E] Resistance to *M. graminicola.*
[F] Commercial varieties developed in Bangladesh.
[H] Commercial varieties developed in Nepal.
[a] Refers to Actual RF {final nematode populations/initial inoculum of 10 eggs/cc soil.

(Table 4.4 Continued)

[b] Refers to percentage of RF as compared to that of susceptible check, which was considered 100%.

[c] The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared susceptible check.

[d] Refer to root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled).

[e] Refers to percentage of RGS as compared to that of susceptible check, which was considered 100%.

[f] Refers to rank converted to a scale of 1-9.

[g] Refers to score = RF rank^2 + RGS rank^2 .

[h] Refers to Resistant Index: Immune = < 2.0, Highly resistant =<4.0, Resistant = < 18, Intermediate = <71, susceptible = < 98 and highly susceptible = > 99.

Table 4.5: Results of repeated evaluation of rice varieties/germplasm which exhibited < 3.0 RGS ratings and <10 RF values in the initial evaluation greenhouse tests. All materials were inoculated with Nepalese isolates of *M. graminicola* (NP 50).

	RF			RGS			RI	
Varieties	Actual[a]	%of ck[b]	Rank[c]	Actual[d]	%of ck[e]	Rank[f]	Score[g]	Reaction[h]
Ramani	428.0	189.3	9	7.8	91.8	9	162	HS
Futuje	176.0	77.8	9	3.8	44.7	7	130	HS
Mansala	409.0	180.9	9	4.5	52.9	7	130	HS
Baram Katika	160.8	71.1	9	2.5	29.4	6	117	HS
IR	835.5	15.7	3	2.5	29.4	6	45	MR
S. Masino	35.0	15.4	3	4.3	50.6	7	58	IM
Baram Kartika	45.0	19.9	3	4.0	47.1	6	45	MR
IR 38	94.0	41.5	6	2.5	29.4	6	72	IM
POBRRO 10	71.0	31.4	5	2.5	29.4	6	61	IM
BH 1442	104	46	6	3.5	41.2	6	72	IM
Bonnet 73	430.0	190.2	9	4.5	52.9	7	130	HS
Labelle	226.0	100.0	9	8.5	100.0	9	162	HS

P = 0.0012

[a] Refers to Actual RF {final nematode populations/initial inoculum of 10 eggs/cc soil.

[b] Refers to percentage of RF as compared to that of susceptible check, which was considered 100%.

[c] The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared susceptible check.

[d] Refer to root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled).

[e] Refers to percentage of RGS as compared to that of susceptible check, which was considered 100%.

[f] Refers to rank converted to a scale of 1-9.

[g] Refers to score = RF rank^2 + RGS rank^2 .

[h] Refers to Resistant Index: Immune = < 2.0, Highly resistant =<4.0, Resistant = < 18, Intermediate = <71, susceptible = < 98 and highly susceptible = > 99.

Table 4. 6: Root galling severity (RGS) ratings and reproductive factors (RF) resulted from inoculating commercial wheat varieties/parental lines developed by Agricultural Systems in Nepal with *M. graminicola*, isolate NP 50.

Varieties	RF			RGS			RI	
	Actual[a]	%of ck[b]	Rank[c]	Actual[d]	%of ck[e]	Rank[f]	Score[g]	Reaction[h]
BW 1040	36.5	217.3	9	6.8	67.2	9	162	HS
BAW 7647	8.0	47.6	6.0	6.0	76.9	8	100	HS
K4	9.0	53.6	7.0	7.5	96.2	9	130	HS
Bl 1022	8.5	50.6	7.0	6.5	83.3	9	130	HS
NL 644	5.8	34.5	5.0	5.5	70.5	8	89	HS
Shatabdi	15.0	89.3	9.0	3.5	44.9	6	117	HS
Gourab	46.5	276.8	9.0	8.0	102.6	9	162	HS
Sourav	65.8	391.7	9.0	8.0	102.6	9	162	HS
Protiva	20.5	122.0	9.0	7.3	93.6	9	162	HS
Sonalika	10.3	61.3	8.0	5.8	74.4	8	128	HS
Kalayansona	52.5	312.5	9.0	8.0	102.6	9	162	HS
Aghrani	15.3	91.1	9.0	7.0	89.7	9	162	HS
Kanchan	17.5	104.2	9.0	8.5	109.0	9	162	HS
Barkat	26.3	156.5	9.0	8.5	109.0	9	162	HS
Ananda	30.5	181.5	9.0	6.0	76.9	9	162	HS
Akabar	7.0	41.7	6.0	6.3	80.8	9	117	HS
CIGM 90-483-4Y -5B-OY-68-OPR	9.8	58.3	7	5.8	74.4	8	113	HS
CIGM 90.455-2Y -1M-OPR-1B-OPR	37	220.2	9	5.3	67.9	8	145	HS

(Table 4.6 contiinued.)

Opata	9.5	56.5	7	5.5	70.5	8	113	HS
Brikuti	16.8	100	9	7.8	100	9	162	HS
Pavon 76	17.5	47.3	6	8.5	134.9	9	117	HS
BAW 272	20.5	55.4	7	7.3	115.9	9	130	HS
BAW 805	30.5	82.4	9	6	95.2	9	162	HS
CM 64224-5Y-1M	46.5	125.7	9	8	127.0	9	162	HS
BAW 378	8.5	23	4	6.5	103.2	9	97	HS
BAW 560	17.5	47.3	6	7.5	119.0	9	117	HS
BAW 824	52.5	141.9	9	8.0	127.0	9	162	HS
CM 84323-C-2Y-OB	10.8	29.2	4	7	111.1	9	97	S
CM 61949-13Y-1M-2Y-								
1M-1Y-1M-OY-1Y	26.3	71.1	8	8.5	134.9	9	145	HS
CM 37705-G-2Y-3M								
-1Y-OM-47Y-OB-19	10.3	27.8	3	5.8	92.1	9	90	S
CM 47046-10M-6Y-16M-								
1Y-1Y-1M-OY-2B	65.8	177.8	9	8	127	9	162	HS
BAW 972	15	40.5	5	3.5	55.6	7	74	S
BAW 284	5.8	15.7	2	5.5	87.3	9	85	S
BD(DIN) 8875-ODI-08D-	I15.3	41.4	5	7.0	111.1	9	106	HS
5D -BCN (Kauz)	7	18.9	2	6.3	100	9	85	S
BD (ISD) 253-63150-								
0ISD-0ISD-RC7-0ISD	36.5	98.6	9	6.8	107.9	9	162	HS
Brikuti	37	100	9	6.3	100	9	162	HS
P = 0.0001								

(Table 4.6 continued)

[a] Refers to Actual RF {final nematode populations/initial inoculum of 10 eggs/cc soil.

[b] Refers to percentage of RF as compared to that of susceptible check, which was considered 100%.

[c] The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared susceptible check.

[d] Refer to root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled).

[e] Refers to percentage of RGS as compared to that of susceptible check, which was considered 100%.

[f] Refers to rank converted to a scale of 1-9.

[g] Refers to score = RF $rank^2$ + RGS $rank^2$.

[h] Refers to Resistant Index: Immune = < 2.0, Highly resistant =<4.0, Resistant = < 18, Intermediate = <71, susceptible = < 98 and highly susceptible = > 99.

Table 4.7: Root galling severity index (RGS) ratings and reproductive factors (RF) resulted from inoculating commercial wheat varieties/parental lines developed by Agricultural Systems in Bangladesh. with *M. graminicola*, isolate NP 50.

Varieties	RF			RGS			RI	
	Actual[a]	%of ck[b]	Rank[c]	Actual[d]	%of ck[e]	Rank[f]	Score[g]	Reaction[h]
BW 1040	36.5	217.3	9	6.8	87.2	9	162	HS
BAW 7647	8.0	47.6	6	6.0	76.9	8	100	HS
K4	9.0	53.6	7	7.5	96.2	9	130	HS
Bl 1022	8.5	50.6	7	6.5	83.3	9	130	HS
NL 644	5.8	34.5	5	5.5	70.5	8	89	HS
Shatabdi	15.0	89.3	9	3.5	44.9	6	117	HS
Gourab	46.5	276.8	9	8.0	102.6	9	162	HS
Sourav	65.8	391.7	9	8.0	102.6	9	16 2	HS
Protiva	20.5	122.0	9	7.3	93.6	9	162	HS
Sonalika	10.3	61.3	8	5.8	74.4	8	128	HS
Kalayansona	52.5	312.5	9	8.0	102.6	9	162	HS
Aghrani	15.3	91.1	9	7.0	89.7	9	162	HS
Kanchan	17.5	104.2	9	8.5	109.0	9	162	HS
Barkat	26.3	156.5	9	8.5	109.0	9	162	HS
Ananda	30.5	181.5	9	6.0	76.9	9	162	HS
Akabar	7.0	41.7	6	6.3	80.8	9	117	HS
CIGM 90-483-4Y-5B-OY-68-OPR	9.8	58.3	7	5.8	74.4	8	113	HS

(Table 4.7 continued)

CIGM 90.455-2Y-1MOPR-1B-OPR	37.0	220.2	9	5.3	67.9	8	145	HS
Opata	9.5	56.5	7	5.5	70.5	8	113	HS
Brikuti	16.8	100.0	9	7.8	100.0	9	162	HS
Pavon 76	17.5	47.3	6	8.5	134.9	9	117	HS
BAW 272	20.5	55.4	7	7.3	115.9	9	130	HS
BAW 805	30.5	82.4	9	6.0	95.2	9	162	HS
CM 64224-5Y-1M-2M-OY	46.5	125.7	9	8.0	127.0	9	162	HS
BAW 378	8.5	23.0	4	6.5	103.2	9	97	HS
BAW 560	17.5	47.3	6	7.5	119.0	9	117	HS
BAW 824	52.5	141.9	9	8.0	127.0	9	162	HS
CM 84323-C-2Y-1B-3Y-OB -CM 61949-13Y-1M-2Y-1M-1Y-1M-OY-1Y	10.8	29.2	4	7.0	111.1	9	97	S
CM 37705-G-2Y-3M-1Y-1Y-OM-47Y-OB-19	26.3	71.1	8	8.5	134.9	9	145	HS
CM 47046-10M-6Y-16M-1Y-1Y-1M-OY-2B	10.3	27.8	3	5.8	92.1	9	90	S
BAW 972	65.8	177.8	9	8.0	127.0	9	162	HS
BAW 284	15.0	40.5	5	3.5	55.6	7	74	S
BD(DIN) 8875-ODI-08D-DI	5.8	15.7	2	5.5	87.3	9	85	S
BCN (Kauz)	15.3	41.4	5	7.0	111.1	9	106	HS
BD (ISD) 253-63150-0ISD-0ISD-RC7-0ISD	7.0	18.9	2	6.3	100.0	9	85	S
	36.5	98.6	9	6.8	107.9	9	162	HS

(Table 4.7 continued)

Brikuti	37.0	100.0	9	6.3	100.0	9	162	HS
P = 0.0002								

[a] Refers to Actual RF {final nematode populations/initial inoculum of 10 eggs/cc soil.

[b] Refers to percentage of RF as compared to that of susceptible check, which was considered 100%.

[c] The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared susceptible check.

[d] Refer to root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled).

[e] Refers to percentage of RGS as compared to that of susceptible check, which was considered 100%.

[f] Refers to rank converted to a scale of 1-9.

[g] Refers to score = RF $rank^2$ + RGS $rank^2$.

[h] Refers to Resistant Index: Immune = < 2.0, Highly resistant =<4.0, Resistant = < 18, Intermediate = <71, susceptible = < 98 and highly susceptible = > 99.

Table 4. 8: Results of repeated evaluation of wheat varieties/germplasm which exhibited < 3.0 RGS ratings and <10 RF values in the initial evaluation greenhouse test.

	RF			RGS			RI	
Varieties	Actual[a]	% of ck[b]	Rank[c]	Actual[d]	% of ck[e]	Rank[f]	Score	Reaction[h]
Annapurna 3	23.0	47.9	6	8.3	131.7	9	117	HS
Annapurna 4	22.0	45.8	6	8.3	131.7	9	117	HS
BL 1813	306.0	637.5	9	7.3	115.9	9	162	HS
Brikuti	48.0	100.0	9	7.6	120.6	9	162	HS
BL 1022	25.0	55.6	7	6.2	98.49		130	HS
BL 1887	20.0	44.4	6	5.3	84.19		117	HS
Annapurna 2	22.0	48.9	6	7.5	119.0	9	117	HS
Brikuti	48.0	100.0	9	6.3	100.0	9	162	HS
P = 0.00011								

[a] Refers to Actual RF {final nematode populations/initial inoculum of 10 eggs/cc soil.

[b] Refers to percentage of RF as compared to that of susceptible check, which was considered 100%.

[c] The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared susceptible check,

[d] Refer to root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled).

[e] Refers to percentage of RGS as compared to that of susceptible check, which was considered 100%.

[f] Refers to rank converted to a scale of 1-9.

[g] Refers to score = RF rank^2 + RGS rank^2 .

[h] Refers to Resistant Index: Immune = < 2.0, Highly resistant =<4.0, Resistant = < 18, Intermediate = <71, susceptible = < 98 and highly susceptible = > 99.

Discussion

Host resistance to plant-parasitic nematodes has been describes as the ability of the plant to suppress the development and reproduction of the nematode, which ranges from low, moderate to a high level. A highly resistant plant allows no or trace amount of nematode reproduction, whereas moderately resistant plants allow intermediate level of reproduction (Roberts, 2002). Resistance to root-knot nematodes is generally characterized by the root-galling severity and/or reproductive factors for prevalent isolates of these nematodes. Thus, resistance is a relative term and it is very difficult to set a distinct boundary to distinguish plant reaction to the target nematode. Often, resistance to root-knot nematode is determined on the basis of infection severity (root-galling severity (RGS) only and ignoring nematode reproduction efficiency or vise versa. Such evaluation can result in misleading information on the host reaction to the tested nematode (Luzzi et al., 1987).

Past evaluations of rice germplasm to root-knot nematode were based mostly on number of root galls and/or root-galling severity (RGS) index (Taya and Dabour, 2004; Yik and Brichfield, 1979; Roy, 1973). However, host resistance evaluations of Prasad et al. (1986) were based on the number of egg masses. In the present study, POBBRO 10, IR 38, Balamchi and Baram Katika exhibited RGS values of lower than 3.0 in the initial test (Tables 2, 4 and 5), thus these varieties or germplasm could be considered resistant to *M. graminicola* as proposed by Griffin and Grey (1995). However, a number of these varieties exhibited a rather high RF values in repeated tests indicating their susceptible reaction to *M. graminicola*.

Many of the tested varieties in the first experiment can be considered resistant as per Trudgill (1986) treatment, because they exhibited RF factors that were lower than 10% of that the susceptible variety (Labelle). He proposed that germplasm

resistance be indexed against known standard susceptible and resistant controls. However, Roberts and May (1986) concluded that varieties or germplasm could not be considered as resistant unless the calculated reproductive factor (RF = Pf/Pi) of the nematode is less than 1. In the present study, none of the varieties and germplasm tested exhibited RF less than 1, thus they are all susceptible according to the definition of Roberts and May (1986).

Results of this study suggested that determining the reaction of rice germplasm to *M. graminicola* will requires assessing both the RGS and RF values, as there was a lack of correlation between these two factors. Mullin et al. (1991) ranked bean germplasm for resistance to root-knot nematodes based on calculating a Resistance Index (RI) that used both root-galling severity and egg mass production [RI= root galling severity rating^2 + egg mass production rating^2]. However, determining the actual number of eggs is more reliable than estimating egg mass production in assessing nematode reproduction (Hussey and Janseen, 2002). Estimation of egg masses in *M. graminicola* in rice and wheat is not possible as eggs are laid inside the root cortex and often difficult to observe intact egg sacs. Thus, a modified reaction scale based on the numbers of eggs produced on roots and on root-galling severity was employed in this study for characterizing the reaction of rice and wheat to *M. graminicola*. However, the method is arbitery and other alternative assessment methods are warranted.

Recent genetic and molecular discoveries indicate that host-plant resistance genes often occur as clusters of genes in a multi-gene family or as multiple allelic series at a disease resistance locus, and in some cases as combination of both "multi-gene" forms (Hammond-Kosack and Jones, 1997). Such complex gene arrangements can also have intra-locus variation. Gene clusters have been identified and characterization in a number of plant-patho systems as factors involved in the defense

against bio-trophic fungi, bacteria, viruses, nematodes and certain insects (Hulbert, 1997). For example, the Mi gene conferring resistance to the southern root-knot nematode (*M. incognita*) belongs to a major class of resistance genes that encodes protein-containing nucleotide binding sites (NBS) and leucin-rich repeats (LRR) and also confer resistance to bacterial, fungal, and viral pathogens (Hammond-Kosack and Jones, 1997; Milligan et al., 1998). Such broad-spectrum resistance might also be achieved through manipulation of defense signaling components and NPRI gene in *Arabidopsis. NPR1* has emerged as a good candidate to provide broad-spectrum resistance, which appears to regulate defense gene transcription through a mode of action (McDowell and Woffenden, 2003) and a gene similar to NPRI has been also observed in rice (Chen et al., 2001). Rice was reported to have about 600 nucleotide binding site leucin-rich repeat (NBS-LRR) resistance genes (Jianta et al., 2002). Since genes within a single cluster can determine resistance to different pathogens (Michelmore and Meyers, 1998), it was hoped that rice germplasm with identified gene clusters may also serve as sources of resistance to *M. graminicola*. Unfortunately, the 60 rice germplasms with reported resistance to either blast, bacterial blight, insect and/or physio-chemical factors were all found to be susceptible in this investigation to an isolate of *M. graminicola* from Nepal (NP 50). The latter result might be due to lack of resistance genes or alleles to this nematode associated with other resistance gene clusters or to the development of new virulent isolates of the nematode.

All tested rice varieties and/or germplasm against *M. graminicola* in this study allowed moderate to high level of nematode infection and reproduction, suggesting a lack of resistance factor(s) in these materials. These results did not confirm the previously reported resistance to *M. graminicola* in rice germplasm (Yik and Brachifild, 1979; Roy, 1973; Bridge et al., 1990). The contradictory results might be due to differences in the nematode isolates used, experimental conditions and/or

methods of inoculation and evaluation. However, several other studies have also failed to identify rice germplasm with resistance to this nematode (Taya and Dabour, 2004; Chunram, 1981; Rao et al., 1986; Roy, 1973; Prasad et al., 1986). Bonnet 73 and LA 110 were considered resistant to a Louisiana isolate of *M. graminicola,* whereas Dumai, Germ and IR 20 were reported as resistant to an Indian isolate of the same nematode. These materials were all susceptible to a Nepali isolate (NP 50) of *M. graminicola* in this study.

Despite the reported resistance to *M. graminicola* in rice germplasm, breeding rice varieties with useful resistance against this nematode has remained elusive (Plowright et al., 1999). Sorianoo et al. (1999) reported that some accessions of *Oryza longistaminata* and *O. glaberrima,* the wild relatives of *O. sativa,* were resistant to an isolate of *M. graminicola* from the Philippines. However, attempts made in the past to incorporate this resistance source from *O. glaberima* into cultivated *O. sativa* germplasm were not encouraging. Although, *O. glaberrima* was highly resistance to *M. graminicola*, the inter-specific progeny tested did not express the same level of resistance as their resistant parent, indicating a need for further back crossing to get acceptable resistant progenies (Plowright et al., 1999). Thus, there is a need to evaluate other wild rice relatives within the genus *Oryza* for resistance to *M. graminicola*, since this genus has more than 20 wild species (Bonman et al., 1992).

All the commercial wheat varieties/parental lines developed by the National Research Centers of Nepal and Bangladesh were found to be susceptible to an isolate (NP 50) of *M. graminicola* from Nepal (Pokharel et al., 2004b). This may be due to the lack of resistance genes in the tested plants and/or development of virulent nematode populations of this nematode in Nepal. In preliminary tests, the 10 most commonly grown wheat varieties in Nepal exhibited a susceptible reaction to a Bangladesh isolate of this nematode. Similarly, Padgham (2003) reported that the

wheat varieties in Bangladesh including Gaurav, Saurab, Satabdi and Kanchan were susceptible to an isolate of *M. graminicola* from Bangladesh. However, Taya and Dabur (2004) screened 19 wheat varieties and reported that all varieties were moderately resistant to *M. graminicola* isolates from India based on the observed number of galls/root system. The difference in these results could be due to the nematode isolates, varieties used and/or evaluation procedures. There remains a great need to identify a source of high level of resistance and to develop adopted commercial wheat varieties with resistance to *M. graminicola,* specially those to be used in rice-wheat systems in SE Asia. It was previously reported that wheat could maintain high population densities of *M. graminicola* between two rice crops (Padgham et al., 2004; Gaur and Sharma, 1999). Under lowland production conditions, wheat is grown in the winter season in the same field after rice, thus the susceptibility or resistance of wheat varieties planted can play a role in the severity of infection and damage of *M. graminicola* on the succeeding rice crop. Since none of the wheat germplasm tested was found to be resistant to *M. graminicola*, rice-wheat systems in Nepal and Bangladesh seems vulnerable to damage by *M. graminicola.*

Generally, higher RGS ratings and RF values were observed when apparently resistant germplasm identified in initial tests was re-evaluated. The contradictory results obtained may be due to the difference in environmental conditions (season the experiment was conducted) or the inherent variability of the experimental methods. Since the experiments were conducted following the same protocol in different seasons, it is most likely that the difference in the results obtained was due to the season of year the experiment was conducted. Environmental factors including light intensity and temperature are known to effect root-knot nematode infection and severity, where cold temperature will slow root-knot nematode development and high temperature will increase reproduction and may also alter the resistant host response

(Hussey and Janseen, 2002). In addition, it is possible that resistance genes in rice against *M. graminicola* are temperature sensitive. The Mi gene conforming resistant against *M. incognita, M. arenaria* and *M. javonica* in tomato (Messaguer et al; 1991; Ho et al; 1992) is sensitive to heat and is not effective at temperature above 28^0 C (Williamson, 1998). Soriano et al. (2000) reported that low nitrogen content of sandy soils might also increase the susceptibility of plants to nematode damage and also reported that the tolerance level of rice cultivars to *M. graminicola* vary under different water management systems. In the present study, uniform nitrogen and water management were employed in all the tests conducted.

Generally, higher RF values for *M. graminicola* were observed on rice than on wheat. The higher reproduction of the nematode in rice might be due to either the genetic make-up of the plants and/or available root masses for nematode growth and reproduction. Rice has a greater root-mass than wheat, thereby supporting higher nematode reproduction. Elkins et al. (1979) reported that a large rooted plants will allow more nematode reproduction and undergo more damage than a small rooted plants, but they also argued that greater root-length will provide more invasion sites for the nematode and wheat is known to generally have longer roots than rice. Soomro and Hague (1992) stressed the importance of the genetic make-up of plants in determining feeding behavior and reproduction of the nematode. Results of the variety x isolate interaction in rice and wheat (Pokharel et al., 2005) provided further supports to the hypothesis that genetic make-up of plants plays greater role in the reproduction of *M. graminicola* in rice than in wheat. Similar results of higher reproduction of *M. graminicola* in rice than wheat were observed by Gaur and Sharma (1999). Crop rotations that include resistant varieties, poor-hosts, or non-hosts can be utilized as an effective strategy for reducing populations densities of plant-parasitic nematodes, but it may require the removal of the susceptible crop from the rotation for

at least 2 to 4 years (Bridge, 1998). *M. graminicola* was reported to have more than 98 host plants that includes cultivated plants and weed (McGowon and Longdan, 1989). Thus, crop selection and weed management in a rice-based rotation seems crucial for the management of this nematode. In addition, this nematode was reported to be a serious problem in onions grown in rice-based rotation in the Philippines as well as other crops rotated with rice (Gergon et al., 2003).

The findings of this investigation documented the high susceptibility of rice and wheat germplasm to *M. graminicola.* Thus, breeders concerned with developing high yielding varieties of rice and/or wheat should consider screening the developed varieties against this nematode before release and to also incorporate factors effective against this nematode, if possible. Due to lack of resistance in rice and wheat, the reaction to this nematode of other crops grown in rice-wheat based rotations needs investigation in order to design an effective crop rotation strategies.

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Overall Summery and Conclusions

The rice root-knot nematode (*Meloidogyne graminicola)* is an important pathogen, has a wide host range, and is widely distributed throughout SE Asia, including Nepal. It causes significant damage to cereals grown in SE Asia, especially to rice. In the current study, *Meloidogyne graminicola* was the only root-knot nematode species identified from 33 rice-wheat fields in Nepal representing the hills and Terai production regions. Results of morphometric measurements of J2, perineal pattern analysis of mature females, symptoms production in roots, host range tests, aggressiveness of isolates and the amplification, sequencing and analysis of internally transcribed spacer regions (ITS) suggested that all the isolates collected were *M. graminicola.* However, minor variability was observed in the above measured characters within and among the isolates studied, especially in the dimension of J2 and symptoms exhibited on susceptible germplasm. All the Nepalese isolates and one isolate each from Florida and Bangladesh formed the same clade based on ITS sequences. This clade was different from clades of other *Meloidogyne* species included in this study for comparison except that of *M. trifoliophila*. However, variability in the ITS sequences was observed among the Nepalese isolates resulting in the recognition of two distinct haplotypes, one representing the isolates collected from the hill region and the other representing those collected from the Terai production region. No correlation was observed between the ITS sequences and morphological, host range and aggressiveness characteristics of the isolates.

The rice cultivars Labelle and LA 110 were susceptible to all the Nepalese isolates of *M. graminicola.* However, there was significant variability in the aggressiveness of these isolates on both varieties. A significant germplasm by isolate

interaction was observed among *M. graminicola* isolates on rice (16 isolates and 8 germplasms) and wheat (8 isolates and 8 germplasms). Generally rice germplasms exhibited higher root-galling severity and reproduction of the nematode as compared to wheat. However, same isolate of the nematode infected rice and wheat which are grown in different environments.

Efficient screening protocols are needed for rapid and accurate germplasm evaluation for resistance to nematodes. Thus, several greenhouse experiments were conducted to develop an efficient protocol for assessing the reaction of rice and wheat to the rice root-knot nematode. The effects 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 were determined. Based on the results of these tests, the protocol adopted for screening rice and wheat germplasm for resistance to *M. graminicola* consisted of inoculating seeds planted in pasteurized soil with 2 or 10 eggs/cc soil (1000 or 5000 eggs/10-cm pot) and incubation for 60 days in greenhouse at 25 C. A lack of correlation between the root-galling severity index and RF values was observed in rice, suggesting the need to use both parameters in assessing the reaction of rice to *M. graminicola*. Higher RF values were obtained in tests conducted in summer months as compared to those conducted in winter months. The latter suggested the effect of temperature and light intensity on the observed host-parasite relationship.

A total of 156 commercial rice varieties and promising germplasm obtained from the International Rice Research Institute (IRRI) and the National Agriculture Centers of Nepal and Bangladesh and also 74 wheat varieties or parental breeding lines obtained from National Agricultural Centers of Nepal and Bangladesh were evaluated for resistance to *M. graminicola* in the greenhouse. The result indicated that all the commercial varieties and promising germplasms of both rice and wheat tested

were susceptible to *M. graminicola*. However, their level of susceptibility varied as measured by both RGS and RF values.

To understand the variability among isolates of *M. graminicola* in different geographic areas, 3 isolates each from Nepal, Bangladesh, India and one isolate from Florida (USA) were compared using traditional and molecular tools. The results of this study revealed that the isolates collected from different geographic regions exhibited similar variability in larval measurements, perineal pattern, and ITS sequences as that found among the Nepalese isolates. However, the results obtained on the aggressiveness and host range of these 10 isolates confirmed the existence of two races in *M. graminicola* that could be separated by their pathogenicity to rice and wheat. The isolate from Florida, USA did not infect or reproduced only poorly in rice, whereas all the other 9 isolates were highly pathogenic in rice, and all 10 isolates severely infected wheat.