GREENHOUSE EVALUATION OF RICE AND WHEAT GERMPLASMS FOR RESISTANCE TO *MELOIDOGYNE GRAMINICOLA* WITH COMMENTS ON EVALUATION INDICES AND PROPOSAL OF A NEW ONE

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Summary. The root-knot nematode (*Meloidogyne graminicola*) is a major constraint to the productivity of rice-wheat production systems in South-East Asia. The use of host resistance is the best possible solution to this problem. However, information on host resistance in rice and wheat to this nematode is limited. Thus, 96 rice cultivars and 59 selected rice entries, with known sources of resistance to other stresses, were evaluated for resistance to Nepali isolates of the nematode. Similarly, the reaction to infection by *M. graminicola* of 74 wheat cultivars and promising breeding lines was also assessed. Ten seeds of rice or wheat were sown per 10-cm clay pot (replicated four times), filled with pasteurized soil (30 minutes at 60 °C) and inoculated with 10 eggs of *M. graminicola*/cm^3 soil. The pots were maintained in a greenhouse at 25 ± 3 °C, irrigated daily, and fertilized once a week. After 60 days, the roots were washed free of soil and root-galling severity (RGS) was rated on a scale of 1 (no visible galls, healthy roots) to 9 (>75% of roots galled). Eggs were then extracted from roots by blending them in 1% sodium hypochlorite solution for 3.5 minutes and sieving the suspension through a 100-mesh sieve nested over a 500-mesh sieve. The suspension from the 500-mesh sieve was collected, nematode eggs and juveniles counted, and the reproductive factor (RF) calculated by dividing total number of eggs and juveniles by total inoculum used (Pf/Pi). The RGS ratings and RF values of the nematode were converted into a new index, the reaction index (RI), derived from the RG ratings and RF values. This reaction index was used to separate germplasm lines or cultivars into immune, highly resistant, resistant, intermediate, susceptible and highly susceptible categories. The results suggested that all the commercial rice and wheat cultivars obtained from Nepal, Bangladesh, and international centres (IRRI and CIMMYT) were susceptible to *M. graminicola* isolates from Nepal. Rice germplasm sources having resistance genes to other pests, diseases, and physiological stresses exhibited greater variability in their reaction as compared to commercial cultivars.

Key words: *Oryza sativa*, resistance, rice root-knot nematode, screening, *Triticum aestivum*.

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

Treating *M. graminicola* infested fields increased rice yield by more than 30% in Nepal by soil solarization (Duxbury, 2002) and 16-31% by carbofuran in Bangladesh (Padgham, 2003). However, the yield loss caused by this nematode in greenhouse tests was much higher (31-97%), depending upon the initial inoculum levels (Sharma-Poudyal *et al.*, 2005). Unfortunately, the use of nematicides and solarization are not reliable options for the control of nematodes in rice. Moreover, growers are generally not sufficiently aware of nematode infection and potential yield losses for the high cost of such control options to be justified. Crop rotation, an effective and sustainable nematode management option, may not be feasible in South-East Asian countries due to the limited availability of land, seasonal flooding, and the high priority for growers to produce rice.

Thus, the use 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 information is available on the reaction of rice cultivars to this nematode and limited efforts have been devoted to breeding resistant rice cultivars (Bridge *et al.*, 2005). Similarly, very limited information is available on the host-parasite relationships of this nematode in wheat. Such information would be useful for designing appropriate crop rotations and for the possible identification of adapted and resistant germplasm for use in a breeding programme. Thus, the aim of this study was to improve the screening protocol and to evaluate the most commonly grown commercial rice and wheat cultivars, alongside promising lines and accessions, for their reaction to isolates of *M. graminicola* from Nepal.

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MATERIALS AND METHODS

A total of 156 rice and 74 wheat cultivars and selected germplasm were tested against *M. graminicola* in a greenhouse at the New York State Agricultural Experiment Station, Geneva, New York State, USA, during 2003-04. Twenty-one commercial rice cultivars were obtained from the International Rice Research Institute (IRRI), Los Baños, Philippines. Also, 38 and 34 commercial rice cultivars were obtained from Nepal and Bangladesh, respectively, through the International Maize and Wheat Improvement Center (CIMMYT) regional offices of the respective countries. In addition, 57 rice accessions that had been characterized for resistance to blast, bacterial blight, insect damage, adverse physio-chemical conditions and rice root-knot nematode (*M. graminicola*) were also obtained from IRRI. Also, two resistant rice cultivars (Bonnet 73, and LA 110) and one susceptible cultivar (Labelle) to a Louisiana isolate of *M. graminicola* (Yik and Birchfield, 1979), and a commercial cultivar (Cordie) were obtained from the Small Grain Repository Center, Dale Bumpers, Georgia, USA. Similarly, 24 and 39 commercial wheat cultivars and promising breeding lines were also obtained from the CIMMYT offices in Nepal and Bangladesh, respectively. Some local wheat accessions were obtained from the Plant Pathology Laboratory in Geneva, USA.

All the rice and wheat cultivars and lines were evaluated for their reaction to *M. graminicola* in different sets of tests. Each test generally included twenty rice or wheat lines and was evaluated using the same protocols and nematode isolates. The isolates of *M. graminicola* used were obtained from rice fields in Nepal and were characterized by Pokharel *et al.* (2007) and maintained in a greenhouse on the susceptible rice cv. Mansuli or barnyard grass (*Echinochloa crus-galli* L.) (Pokharel *et al.*, 2004a). The highly virulent isolate of *M. graminicola* from Nepal (NP 50) (Pokharel *et al.*, 2007) was included in the tests as a highly virulent isolate can discriminate genotypes with the highest level of resistance (Hussey and Janseen, 2002). All lines exhibiting a reproductive factor (RF) of <1.0 and root-galling severity (RGS) <3.0 in the first tests were re-evaluated using three different isolates of *M. graminicola* (NP 29, NP 30 and NP 50).

In all tests, entries were arranged in the greenhouse in a completely randomized design with four replicates. Each replicate consisted of a sterilized 10-cm-diameter pot filled with 500 cm^3 of pasteurized (60 °C for 30 minutes) mineral clay loam soil of pH 6.5. Ten rice or ten wheat seeds were sown per pot, inoculated with 10 eggs of *M. graminicola*/cm^3 soil and covered with a soil-peat moss mixture. All pots were maintained at 25 °C for 60 days and received daily watering and weekly fertilization with approximately 2.5 grams of NPK (10-10-10) fertilizer per litre of water. The tests were repeated once unless a cultivar or line showed a susceptible or highly susceptible reaction, when the cultivar or line was discarded.

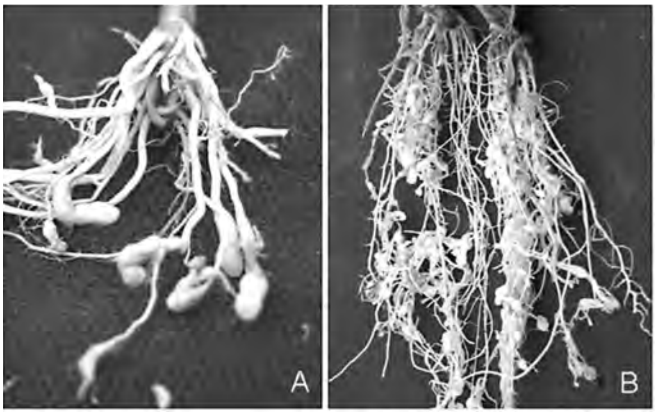

Fig. 1. Root-knot symptoms on the roots of susceptible rice cv. Labelle (A) and wheat cv. Brikuti (B) following inoculation with *Meloidogyne graminicola* in greenhouse tests.

After 60 days, the plants were uprooted, the roots were washed free of soil and the severity of root-galling (RGS) was determined on a 1-9 scale based on the proportion of roots galled: 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 the roots galled (Mullin *et al.*, 1991). Nematode eggs were then extracted by blending the roots of all ten plants per pot for 3 minutes in a 1% sodium hypochlorite solution. The suspension was then sieved through a 100-mesh sieve nested on a 500-mesh sieve. Then the content on the 500 mesh sieve was collected in a beaker, and the volume adjusted to 100 ml. Dilution series were then prepared (if needed) and the number of eggs in a 10 ml aliquant per sample was recorded under a dissecting microscope (40×). The reproductive factor RF (= total number of eggs and juveniles extracted divided by the number of eggs used to infest the soil/pot) was calculated for each nematode isolate for each cultivar or line tested.

The host reaction was assessed as proposed by Mullin *et al.* (1991), with some modification as described later in this section since egg masses of this root-knot nematode species are deposited inside the roots and are difficult to extract. The calculated RF was converted to a 1-9 scale based on its value as a percentage of that of the susceptible check cultivar (Labelle in rice and Brikuti in wheat, Fig. 1). The 1-9 scale was 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 cultivar. Since the RGS ratings of the susceptible checks varied between tests, the observed RGS ratings of the cultivars and lines under test were also converted to percentages of that of the susceptible checks, and the calculated % RGS ratings were then converted to a similar 1-9 scale. The reaction indices of tested material were determined following the 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; highly resistant (HR), RI = 2.1 to 4.0; resistant (R), RI = 4.1-18; moderately resistant (MR), RI = 18.1-50; intermediate (IM), RI = 51-71; susceptible (S), RI = 72-98; and highly susceptible (HS), RI > 99.

Data for all parameters used in the tables were analyzed by ANOVA, using Proc GLM, LSD (P = 0.05) for mean comparison of RF, and NPAR1WAY (Kruskal-Wallis test), non-parametric one-way ANOVA (P = 0.05 level) for the rest of the variables by SAS Enterprise guide, SAS, Institute. The P value expressed in each table is from the Kruskal-Wallis test, an equivalent to "Wilcoxon scores", and is the test of the homogeneity by "Chi-square" test (McDonald, 2009).

RESULTS

Rice

The actual RF values for the commercial rice cultivars obtained from IRRI ranged from 4.3 to 252.5 (Table I). The cultivars POBRRE 4 (4.3), POBRRE 10 (5.0) and IR 38 (7.4) had the lowest actual RF values, and IR 66 (252.5), IR 24(202.0) and IR 64 (146.0) had the highest actual RF values. The actual RGS values for the same lines ranged from 2.3 to 9.0, with POBRRE 10 (2.3), IR38 (2.5) and IR 32 (3.2) having the lowest RGS ratings, and IR 46 and IR 64 (both 9.0), and IR 8 and Labelle (both 8.5) the highest RGS ratings. However, two (POBRRE 10 and IR 38) and five (POBRRE 4, IR 32, IR 36, IR 50 and IR 88) cultivars were moderately resistant and intermediate, respectively, according to the calculated reaction index (RI) (Table I). Interestingly, the cultivar IR 20, reported to be resistant, was found to be susceptible in this study. Generally, RF values did not correlate with the RGS ratings.

The rice cultivars from Bangladesh had actual RF values ranging from 2.8 to 146, where BARI 24 (2.8), BARI 10 (4.8) and BARI 39 (5.8) had lower actual RF values and BARI 29 (146.0), BARI 22 (145.0) and BARI 25 (108.5) had the greatest actual RF values. Actual RGS ratings ranged from 3.5 to 9, where BARI 27 (3.5),

Table I. Reproduction factors (RF) of *Meloidogyne graminicola* isolate NP 50 and root-galling severity (RGS) rating, on commercial rice cultivars developed by the International Rice Research Institute (IRRI), and resistance index (RI) of these cultivars to the nematode.

Cultivar	RF			RGS			RI	
	Actual[a]	% of check[b]	Rank[c]	Actual[d]	% of check[e]	Rank[f]	Score[g]	Host reaction[h]
IR 22	116.0	79.5	9	7.5	88.2	9	162	HS
IR 32	35.8	24.5	4	3.8	44.7	7	65	IM
IR 5	78.3	53.6	7	7.8	91.8	9	130	HS
IR 24	202.0	138.4	9	8.0	94.1	9	162	HS
IR 28	95.3	65.3	8	8.3	97.6	9	145	HS
IR 8	69.3	47.5	6	8.5	100.0	9	117	HS
POBRRE 10	5.0	13.4	3	2.3	27.1	6	45	IM
POBRRE 4	4.3	2.9	2	4.8	56.5	7	53	IM
IR 36	9.0	6.2	2	5.5	64.7	8	68	IM
IR 50	11.8	8.1	2	5.5	64.7	8	68	IM
IR 38	7.4	14.6	3	2.5	29.4	6	45	IM
IR 88	23.0	15.8	3	5.0	58.8	7	58	IM
IR 42	54.0	37.0	5	6.0	70.6	8	89	S
IR 20	15.0	10.3	3	7.0	82.4	9	90	S
IR 26	12.0	8.2	2	8.0	94.1	9	85	S
IR 60	46.0	31.5	5	8.0	94.1	9	106	HS
IR 54	79.0	54.1	7	8.0	94.1	9	130	HS
IR 46	10.0	6.8	2	9.0	105.9	9	85	S
IR 64	146.0	100.0	9	9.0	105.9	9	162	HS
IR 66	252.5	17.5	3	6.5	76.5	9	90	S
IR 62	68.0	46.6	6	7.5	88.2	9	117	HS
Labelle[i]	147.2	100.0	9	8.5	100.0	9	106	HS
F/Chi-square value	76.18	76.0	61.507	21.0	62.43	56.6	76.06	
P =	<000**	0.001*	0.001*	<0.000*	>0.00*	<0.0000*	0.0001*	

*Kruskal-Wallis test and ** ProcGLM (SAS). [a]Actual RF = final nematode populations/initial inoculum of 10 eggs/cc soil. [b]Percentage of RF as compared to that of the susceptible check. [c]The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared to the susceptible check. [d]Root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled). [e]Percentage of RGS as compared to that of the susceptible check. [f]Rank converted to a scale of 1-9. [g]Score = RF rank^2 + RGS rank^2. [h]Refers to host reaction index: Immune (I) < 2.0, Highly resistant (HR) < 4.0, Resistant (R) < 18, Intermediate (IM) < 71, susceptible (S) < 98 and highly susceptible (HS) > 99. [i]Susceptible check included for comparison. The reaction of the other commercial cultivars developed by the International Rice Research Center (IRRI) was not known.

BARI 26 (4.5) and BARI 19 (4.8) had the lowest actual RGS ratings, and Labelle (9.0), BARI 7 and BARI 8 (8.8) had the highest RGS ratings (Table II). The reportedly resistant LA 110 rice showed a susceptible reaction to this nematode (isolate NP 50).

Similarly, the cultivars obtained from Nepal had actual RF values ranging from 5.0 to 96.8. Rice germplasm BH 1442 (5.0), Radha-9 and Radha-12 (both 5.3) had the lowest actual RF values, and BP1-3-2 (96.8), CH 45 (34.0), and Ghaya (29.5) had the highest RF values. Actual RGS values ranged from 3.0 to 9.0, where Chaite 2 (3.0) had a low RGS value but many cultivars had the highest (9.0) RGS rating (Table III). Again, the resistant rice, LA 110, exhibited a susceptible reaction to the nematode isolate from Nepal (isolate NP 50). For the rice cultivars selected for their potential resistance to other

Table II. Reproductive factors (RF), root-galling severity (RGS) rating and resistance index (RI) resulting from inoculation of rice cultivars developed by the Agricultural Systems in Bangladesh with *M. graminicola,* isolate NP 50.

Cultivar	RF			RGS			RI	
	Actual[a]	% of check[b]	Rank[c]	Actual[d]	% of check[e]	Rank[f]	Score[g]	Host 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
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
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	11.5	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	162	HS
BARI 40	14.0	90.3	9.0	7.2	120.0	9	162	HS
LA 110	23.5	47.0	6	7.4	82.2	9	117	HS
Labelle[i]	15.5	100.0	9.0	9.0	100.0	9	162	HS
F/Chi-square value	14.24	16.5	10.04	23.10	120.3	23.3	75.3	
P =	0.0757**	0.056*	0.0756	0.023*	0.002*	0.0016*	0.003*	

*Kruskal-Wallis test and ** ProcGLM (SAS). [a]Actual RF {final nematode populations/initial inoculum of 10 eggs/cc soil. [b]Percentage of RF as compared to that of the susceptible check. [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]Root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled). [e]Percentage of RGS as compared to that of the susceptible check. [f]Rank converted to a scale of 1-9. [g]Score = RF rank^2 + RGS rank^2. [h]Refers to host reaction index: Immune (I) < 2.0, Highly resistant (HR) < 4.0, Resistant (R) < 18, Intermediate (IM) < 71, susceptible (S) < 98 and highly susceptible (HS) > 99. [i]Susceptible check included for comparison. All other cultivars developed and or recommended for cultivation by Bangladesh Agricultural Research Institute, Bangladesh, are not known for their reaction to *M. graminicola*.

pathogens, insects or environmental stress conditions, the actual RF ranged from 3.5 to 187.8, and the actual RGS ranged from 1.8 to 9.0. The cultivar Futuje had the lowest RF value, but the cultivar Balamchi had the lowest RGS rating (Table IV).

Wheat

Wheat germplasm from Nepal, Bangladesh and few entries from the USA showed similar reactions to an isolate (NP 50) of *M. graminicola* from Nepal. The actual RF values of the nematode ranged from 0.3 (BL 1887) to 87.0 (BAW 65) and the actual RGS values ranged

Table III. Reproductive factors (RF), root-galling severity (RGS) rating and resistance index (RI) resulted from inoculation of rice cultivars developed by the Agricultural Systems in Nepal with *M. graminicola,* isolate NP 50.

Cultivar	RF			RGS			RI	
	Actual[a]	% of check[b]	Rank[c]	Actual[d]	% of check[b]	Rank[f]	Score[g]	Host reaction[h]
IR5/872	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	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
Bp1 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	29.0	132.4	9	6.3	105.0	9	162	HS
Bp1- 3-2	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	100.0	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	9.0	151.7	9	97	S
NR 601-11-9	13.0	59.4	6	9.0	155.0	9	117	HS
Pusha 834	26.8	122.4	9	9.0	155.0	9	162	HS
Labelle	21.9	100.0	9	9.0	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	110.0	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	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
LA110	13.0	59.4	6	9.3	155.0	9	117	HS
Labelle[i]	22.0	100.0	9	6.0	100.0	9	162	HS
F/Chi-square value	23.2	86.2	26.6	21.1	78.3	22.	69.5	
P =	0.001**	0.003*	0.002*	0.0023*	0.003*	0.003*	0.004*	

* Kruskal-Wallis test and ** ProcGLM (SAS). [a]Actual RF = final nematode populations/initial inoculum of 10 eggs/cc soil. [b]Percentage of RF as compared to that of the susceptible check. [c]The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared with the susceptible check. [d]Root-galling severity determined on a scale of 1 (no galls observed) to 9.0 (> 80% of roots galled). [e]Percentage of RGS as compared to that of the susceptible check. [f]Rank converted to a scale of 1-9. [g]Score = RF rank^2 + RGS rank^2. [h]Refers to host reaction index: Immune (I) < 2.0, Highly resistant (HR) < 4.0, Resistant (R) < 18, Intermediate (IM) <71, susceptible (S) < 98 and highly susceptible (HS) > 99. [i] Susceptible check included for comparison. All other cultivars developed and or recommended for cultivation by the Nepal Agricultural Research Council, Nepal, were not known for their reaction to *M. graminicola*.

Table IV. Reproductive factor (RF), root galling severity (RGS) rating and resistance index (RI) resulting from inoculating rice germplasm having resistance genes to other pathogens, insects and physio-chemical stresses with *M. graminicola,* isolate NP 50.

Germplasm	RF			RGS			RI	
	Actual[a]	% of check[b]	Rank[c]	Actual[d]	% of check[e]	Rank[f]	Score[g]	Host reaction[h]
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
Jalmani[A]	15.3	106.9	9	5.5	114.6	9	162	HS
C-5905[A]	10.3	72.0	8	5.3	110.4	9	145	HS
Sano B. Chiya[A]	5.0	17.2	3	7.0	87.5	9	90	S
Ghure[A]	26.3	183.9	9	6.8	141.7	9	162	HS
Bengsar[A]	30.5	61.0	8	5.3	58.9	7	113	HS
Basbarelli[A]	64.0	220.6	9	5.0	62.5	9	130	HS
Belgudi[A]	37.0	127.5	9	7.0	87.5	9	162	HS
Sajani[A]	69.5	239.6	9	8.5	106.3	6	162	HS
Baram Kartika[A]	5.3	10.6	3	3.5	38.9	9	45	IM
Amaghaud[A]	27.0	93.1	9	7.7	96.3	5	162	HS
Balamachi[A]	6.5	13.0	3	1.8	20.0	9	34	MR
Dumai[AE]	7.5	52.4	7	4.8	100.0	9	130	HS
Geram[AE]	16.3	113.9	9	5.8	120.8	9	162	HS
Lamani[B]	16.5	115.3	9	5.0	104.2	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
Jarmani[B]	11.0	76.9	9	6.0	125.0	9	162	HS
Sulidhan Masino[B]	5.3	18.2	3	3.3	41.3	6	45	IM
Balumsan[B]	10.5	36.2	5	5.3	66.3	8	89	S
Phulpata[B]	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
Chengul (Bine) [B]	20.5	70.6	8	7.3	91.3	9	145	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
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
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
Labelle	14.3	100.0	9	4.8	100.0	9	162	HS
Palpalee[C]	10.5	73.4	8	5.3	110.4	9	145	HS
White Atte[C]	40.5	139.6	9	8.5	106.3	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
R 146[C]	57.5	198.2	9	8.3	103.8	9	162	HS
Bageri[C]	16.5	115.3	9	5.0	104.2	9	162	HS
Thulo Achheme[D]	11.8	40.6	6	3.8	47.5	6	72	S
Putuje[D]	3.5	24.4	3	3.3	68.8	8	73	S
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
Paheli[D]	35.3	121.7	9	5.5	68.8	8	145	HS
Garue Ghaiya[D]	14.5	50.0	6	8.0	100.0	9	117	HS
Suga Pankhe[D]	99.5	343.1	9	8.3	103.8	9	162	HS
Jumula-2[D]	10.5	73.4	9	4.3	89.6	9	162	HS
Kanegira[D]	33.0	66.0	7	3.8	42.2	6	85	S
Suga Pankha[D]	54.5	109.0	9	6.0	66.7	8	145	HS
Simtharo[D]	105.5	211.0	9	6.5	72.2	8	145	HS
Dheradun Basmati[D]	130.3	260.6	9	9.0	100.0	9	162	HS
Katakana	73.0	146.0	9	8.0	88.9	9	162	HS
Panbira	16.0	32.0	5	8.5	94.4	9	106	HS
H. kalmi	7.5	15.0	3	5.5	61.1	9	90	S
M. Bati	5.8	11.6	3	7.8	86.7	9	90	S
Dular	10.3	20.6	4	7.0	77.8	9	97	S
Deharil	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[i]	50.0	100.0	9	9.0	100.0	9	162	HS
F/Chi-square value	27.3	69.3	21.3	27.5	72.6	25.3	69.7	
P =	0.001**	0.0023*	0.001*	0.001*	0.005*	0.64*	0.005*	

*Kruskal-Wallis test and ** ProcGLM (SAS). [A]Resistant to bacterial blight. [B]Resistant to blast. [C]Resistant to insects. [D]Resistant to physio-chemical stresses. [E]Resistant to *M. graminicola*. [a]Actual RF = final nematode populations/initial inoculum of 10 eggs/cc soil. [b]Percentage RF as compared to that of the susceptible check. [c]The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared to the susceptible check. [d]Refers 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 the susceptible check. [f]Refers to rank converted to a scale of 1-9. [g]Refers to score = RF rank^2 + RGS rank^2. [h]Refers to host reaction index: Immune (I) < 2.0, Highly resistant (HR) < 4.0, Resistant (R) < 18, Intermediate (IM) < 71, susceptible (S) < 98 and highly susceptible (HS) > 99. [i]Susceptible check included for comparison.

from 1.9 (Annapurna 4) to 9.0 (BAW 65) (Table V). Nine cultivars exhibited an intermediate reaction according to the RI utilized in this study. The actual RF for wheat cultivars from Bangladesh ranged from 5.8 (NL 644) to 65.8 (Sourav and 16M-1Y-1Y-1M-OY-2B), and actual RGS ranged from 3.5 (Satabdi) to 8.5 (in several cultivars).

The actual RF values of the US wheat cultivars tested ranged from 11.7 (US 1) to 27.5 (Harus) and the actual RGS values ranged from 5.0 (US 1 and Tacoma) to 7.8 (NY strain 1) (Table VI).

When cultivars and lines of rice and wheat with the lowest RGS (<3.0) and lowest RF (<1.0) from the first test were re-evaluated against *M. graminicola*, similar

Table V. Reproductive factors (RF), root galling severity (RGS) rating and resistance index (RI) resulting from inoculating wheat cultivars and germplasm developed by the Agricultural Systems in Nepal with *M. graminicola* isolate NP 50.

Cultivar/parental lines	RF			RGS			RI	
	Actual[a]	% of check[b]	Rank[c]	Actual[d]	% of check[e]	Rank[f]	Score[g]	Host reaction[h]
Achyut	4.6	34.8	5.0	4.6	51.1	7.0	74	S
BL 1473	13.4	36.4	5.0	6	66.7	8.0	89	S
BL 1923	15.7	42.7	6.0	4.6	51.1	7.0	85	S
Kranti	18.6	50.5	7.0	4.4	48.9	6.0	85	S
Nepal 297	8.9	24.2	4.0	4.8	53.3	7.0	65	S
NL 972	11.1	30.2	5.0	3.2	35.6	5.0	50	IM
Pasang Lamu	13.0	35.3	5.0	3	33.3	5.0	50	IM
Rohini	9.0	24.5	4.0	4.6	51.1	7.0	65	S
RR 21	13.3	36.1	5.0	2.8	31.1	5.0	50	IM
Annapurana1	9.0	24.5	4.0	3.9	43.3	6.0	52	S
Annapurana2	1.5	4.1	2.0	3	33.3	5.0	29	IM
Annapurana3	2.8	7.6	2.0	2.3	25.6	4.0	20	IM
Annapurana4	5.0	13.6	3.0	1.9	21.1	4.0	25	IM
BL1022	3.5	9.5	2.0	2.4	26.7	4.0	20	IM
BL1813	4.5	12.2	3.0	3.3	36.7	5.0	34	IM
BL1887	0.3	0.8	2.0	2	22.2	4.0	20	IM
Up 262	12.8	34.8	5.0	4.6	51.1	7.0	74	S
BL 1473	13.4	36.4	5.0	6	66.7	8.0	89	S
BL 1923	15.7	42.7	6.0	4.6	51.1	7.0	85	S
Chirya-3	10.0	27.2	4.0	4	44.4	6.0	52	S
BAW 982	20.20	54.3	7.0	6.5	72.2	9.0	130	HS
Chirya-1's	8.0	21.7	4.0	6.5	72.2	9.0	97	S
GMAY # 4 CIGM 67-117-3Y-3M-1PR-1M-3PR[i]	10.0	27.2	4.0	5.8	64.4	8.0	80	S
CM-76502-019M-02AL-2Y-8M-OY[i]	10.0	27.2	4.0	4.8	53.3	8.0	80	S
CM 75955-E-4M-3Y-03M -02Y-2B-1Y-OB[i]	17.5	47.6	6.0	7.5	83.3	9.0	117	HS
CM 91220-1ISD-1ISD-0ISD[i]	9.0	24.5	4.0	7	77.8	9.0	97	S
BAW 65	87.0	236.4	9.0	9	100.0	9.0	162	HS
NL 297	9.0	24.5	4.0	7.8	86.7	9.0	97	S
BAW 879	49.0	133.2	9.0	8.3	92.2	9.0	162	HS
BAW 979	6.0	16.3	3.0	7.5	83.3	9.0	90	S
BAW 996	15.0	40.8	6.0	4.8	53.3	7.0	85	S
BAW 989	9.0	24.5	4.0	5.8	64.4	8.0	80	S
BAW 908	10.0	27.2	4.0	3.8	42.2	6.0	52	S
Brikuti[i]	24.6	100.0	8.0	4.8	100.0	7.0	113	HS
F/Chi-square value	49.32	63.2	25.3	24.6	53.03	21.6	54.02	
P value	0.002**	0.003*	0.003	0.043*	0.003*	0.002*	0.001*	

* Kruskal-Wallis test ** Proc GLM. [a]Actual RF = final nematode populations/initial inoculum of 10 eggs/cc soil. [b]Refers to percentage of RF as compared to that of the susceptible check. [c]The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared to the susceptible check. [d]Refers 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 the susceptible check. [f]Refers to rank converted to a scale of 1-9. [g]Refers to score = RF rank^2 + RGS rank^2. [h]Refers to host reaction index: Immune (I) < 2.0, Highly resistant (HR) < 4.0, Resistant (R)< 18, Intermediate (IM) < 71, susceptible (S) < 98 and highly susceptible (HS) > 99. [i]Susceptible check included for comparison. All other wheat cultivars are developed and or recommended for commercial cultivation by the Nepal Agricultural Research Council (NARC), Nepal.

[i]Germplasm. The remaining entries are cultivars.

Table VI. Reproductive factors (RF), root galling severity index (RGS) rating and resistance index (RI) resulting from inoculating wheat cultivars and germplasm developed or recommended by Agricultural Systems in Bangladesh and a few selected wheat lines, with *M. graminicola*, isolate NP 50.

Cultivar/line	RF			RGS			RI	
	Actual[a]	% of check[b]	Rank[c]	Actual[d]	% of check[e]	Rank[f]	Score[g]	Host reaction[h]
BW 1040	36.5	55.5	7	6.8	80.0	9.0	130	HS
BAW 764	10.8	16.4	3	7	82.4	9.0	90	S
K4	9	13.7	3	7.5	88.2	9.0	90	S
Bl 1022	8.5	12.9	3	6.5	76.5	9.0	90	S
NL 644	5.8	8.8	2	5.5	64.7	8.0	68	S
Shatabdi	15	22.8	4	3.5	41.2	6.0	52	S
Gourab	46.5	70.7	9	8	94.1	9.0	162	HS
Sourav	65.8	100.0	9	8	94.1	9.0	162	HS
Protiva	20.5	31.2	5	7.3	85.9	9.0	106	HS
Sonalika	10.3	15.7	3	5.8	68.2	8.0	73	S
Kalayansona	52.5	79.8	7	8	94.1	9.0	130	HS
Aghrani	15.3	23.3	4	7	82.4	9.0	97	S
Kanchan	17.5	26.6	5	8.5	100.0	9.0	106	HS
Barkat	26.3	40.0	5	8.5	100.0	9.0	106	HS
Ananda	30.5	46.4	6	6	70.6	9.0	117	HS
Akabar	7	10.6	3	6.3	74.1	9.0	90	S
CIGM 90-483-4Y-5B-OY-68-OPR	9.8	14.9	3	5.8	68.2	8.0	73	S
CIGM [1] 90.455-2Y-1M-OPR-1B-OPR[1]	37	56.2	7	5.3	62.4	8.0	113	HS
Opata[1]	9.5	14.4	4	5.5	64.7	8.0	80	S
Pavon 76	17.5	26.6	4	8.5	100.0	9.0	97	S
BAW 272	20.5	31.2	5	7.3	85.9	9.0	106	HS
BAW 805	30.5	46.4	6	6	70.6	9.0	117	HS
CM 64224-5Y-1M-2M-OY[1]	46.5	70.7	9	8	94.1	9.0	162	HS
BAW 378	8.5	12.9	3	6.5	76.5	9.0	90	S
BAW 560	17.5	26.6	4	7.5	88.2	9.0	97	S
BAW 824	52.5	79.8	9	8	94.1	9.0	162	HS
CM 84323-C-2Y-1B-3Y-OB[1]	10.8	16.4	3	7	82.4	9.0	90	S
CM 61949-13Y-1M-2Y-1M-1Y-1M-OY-1Y[1]	26.3	40.0	4	8.5	100.0	9.0	97	S
CM 37705-G-2Y-3M-1Y-OM-47Y-OB-19	10.3	15.7	3	5.8	68.2	8.0	73	S
CM 47046-10M-6Y-16M-1Y-1Y-1M-OY-2B[1]	65.8	100.0	9	8	94.1	9.0	162	HS
BAW 972	15	22.8	4	3.5	41.2	6.0	52	S
BAW 284	5.8	8.8	2	5.5	64.7	8.0	68	S
BD(DIN) 8875-ODI-08D-5DI	15.3	23.3	4	7	82.4	9.0	97	S
BCN (Kauz)	7	10.6	3	6.3	74.1	9.0	90	S
BD (ISD) 253-63150-0ISD-0ISD-RC7-0ISD	36.5	55.5	7	6.8	80.0	9.0	130	HS
US Varieties								
US 1	11.7	31.8	5.0	5	55.6	7.0	74	HS
NY Strain	27	73.4	9.0	7.8	86.7	9.0	162	HS
Harus	27.5	74.7	9.0	7	77.8	9.0	162	HS
Tacoma	13.7	37.2	5.0	5.5	61.1	8.0	89	HS
Brikuti[i]	26.8	100.0	9.0	7	100.0	9.0	162	HS
F/Chi-square Value	132.2	53.6	27.3	23.6	56.03	28.3	63.2	
P =	0.001**	0.0002*	0.0003*	0.001*	0.0002*	0.002*	0.0003*	

*Kruskal-Wallis test and ** ProcGLM (SAS). [a]Actual RF = final nematode populations/initial inoculum of 10 eggs/cc soil. [b]Percentage of RF as compared to that of the susceptible check. [c]The RF rank was converted to a severity scale which was calculated as 1 = (0 % reproduction) to 9 (> 70% reproduction) as compared to the susceptible check. [d]Refers 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 the susceptible check. [f]Refers to rank converted to a scale of 1-9. [g]Refers to score = RF rank^2 + RGS rank^2. [h]Refers to host reaction index: Immune (I) < 2.0, Highly resistant (HR) < 4.0, Resistant (R) < 18, Intermediate (IM) < 71, susceptible (S) < 98 and highly susceptible (HS) > 99. [i]Susceptible check included for comparison. All other wheat cultivars are developed and or recommended for commercial cultivation by the Bangladesh Agricultural Research Institute (BARI), Bangladesh.

[i]Germplasm. The remaining entries are cultivars.

variability in their reaction was observed (data not shown). However, the results obtained followed a trend similar to that of the first test. The RF values and RGS ratings of the same nematode isolate on different lines were similar in both tests. The RF values ranged from 35.0 to 409.0 and RGS ratings ranged from 2.5 to 7.5 in the re-tests. The repeated tests were conducted during the summer months, while the previous tests were conducted during the winter months (January to March).

High variability in RF and RGS, both in rice and wheat, was observed. RF and its derivatives (percentage relative to check and rank) as well as RGS rating and its derivatives (percentage relative to check and rank) varied significantly (P = 0.05 level) between the different cultivars of rice and wheat tested.

DISCUSSION

Host resistance to plant-parasitic nematodes has been described as the ability of the plant to suppress development and reproduction of the nematode, which ranges from low through moderate to a high level. Resistance is a relative term, and it is very difficult to set a distinct boundary to distinguish plant reaction to the target nematode. Resistance in plants to root-knot nematodes is generally characterized by the root-galling severity (RGS) ratings and/or reproductive factor (RF) values for prevalent isolates of the nematodes.

In the present study, the rice germplasm POBRRE 10, IR 38 and Balamchi (Tables II, IV) and the wheat germplasm Annapurama3, Annapurama4, BL1022 and BL 1887 (Tabble V) exhibited actual RGS ratings lower than 3.0, so could be considered resistant to *M. graminicola* as proposed by Griffin and Grey (1995). However, some of them had rather high RF values, suggesting that they should not be considered as resistant to *M. graminicola* as they failed to suppress development and reproduction of this nematode. Nevertheless, several of the cultivars tested can still be considered resistant according to Trudgill (1986), because they exhibited RF values lower than 10% of that of the susceptible cultivar Labelle. Trudgill (1986) proposed that germplasm resistance be indexed against known standard susceptible and resistant controls. Unfortunately, all resistant lines or cultivars to *M. graminicola* included in the current study were found to be susceptible. Thus all of the materials tested should be considered susceptible according to the definition of Roberts and May (1986). These authors concluded that a cultivar or line cannot be considered as resistant unless the calculated reproductive factor (RF = Pf/Pi) of the nematode is less than 1, but none of the material tested in this study had RF less than 1. However, this general rule of RF <1 may not be relevant to all root-knot/host combinations. For example, rice on average exhibited six times higher RF values than wheat infected by the nematode isolate used in this study. In the absence of a close correlation between RGS ratings and RF values, the use of both variables was suggested for *M. graminicola* in rice and wheat cultivars (Pokharel *et al.*, 2004b) and in cotton (Luzzi *et al.*, 1987) and bean (Mullin *et al.*, 1991) against *Meloidogyne* spp.

Often, resistance to root-knot nematode is determined on the basis of infection severity (root-galling severity), ignoring nematode reproduction efficiency or vice versa (Roberts, 2002). Such evaluation can result in misleading information on host reaction to nematodes (Luzzi *et al.*, 1987) since the two variables are under the control of independent genetic factors. Root-galling was reported to be governed by nematode-activated chemical release (Trudgill, 1991), while reproduction is governed by host plants (Giebel, 1982). Only RGS (Yik and Birchfield, 1979) or only nematode reproduction (Soriano *et al.*, 1999) was considered in the evaluation of rice germplasm against *M. graminicola* in the past. Mullin *et al.* (1991) ranked bean germplasm for resistance to root-knot nematodes based on a resistance index (RI) of the host reaction 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). Also, estimation of numbers of egg masses in *M. graminicola* in rice and wheat is not possible as eggs are laid inside the root cortex and it is often difficult to extract intact egg masses. Thus, a modified reaction scale based on the numbers of eggs produced on the roots and on root-galling severity was employed in this study while characterizing the reaction of rice and wheat to *M. graminicola*. Because of high variability of and lack of correlation between RGS ratings and RF values, when evaluating the reaction of rice and wheat to *M. graminicola*, we propose the use of a new resistant index (RI). This index is derived from both root gall severity index (RGS) and reproductive factor (RF) and has shown to be much less variable.

Based on this resistance index, all commercial rice cultivars obtained from Nepal, Bangladesh and the International Rice Research Institute were susceptible to a Nepali isolate of *M. graminicola*. This suggests that most of the rice cultivars recommended for cultivation lack resistance or tolerance to this nematode. The risk of nematode damage to rice increases with increase of the inoculum level (Sharma-Poudyal *et al.*, 2005) and crop management practices favourable to the nematode, such as growing susceptible cultivars. Areas with higher nematode population levels need special nematode management programmes. Similarly, all the commercial wheat cultivars/parental lines from Nepal and Bangladesh were found to be susceptible to an isolate (NP 50) of *M. graminicola* from Nepal. This may be due to the lack of resistance genes in the tested plants and/or development of virulent nematode populations in Nepal. In preliminary tests, the ten most commonly grown wheat cultivars in Nepal exhibited a susceptible reaction to a Bangladesh isolate of this nematode. Similarly, Padgham (2003) reported that Gaurav, Saurab, Satabdhi and Kanchan wheat cultivars from Bangladesh were susceptible to an isolate of *M. graminicola* from Bangladesh. However, Taya and Dabur (2004) screened nineteen wheat cultivars and reported that all were moderately resistant to *M. graminicola* isolates from India based on the observed number of galls/root system. The differences in these results could be due to difference in the nematode isolates, cultivars, and/or evaluation procedures used. There remains a great need to identify a source of high level resistance and to incorporate a resistance gene into commercial high yielding cultivars. It was previously reported that wheat could maintain high population densities of *M. graminicola* between two rice crops (Gaur and Sharma, 1999; Padgham *et al.*, 2004). Under lowland production conditions, wheat is grown in the winter season in the same field after rice, thus the susceptibility or resistance of wheat cultivars planted can play a vital 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*, the rice-wheat crop system in Nepal and Bangladesh seems vulnerable to damage by *M. graminicola*, and the identification of resistance sources and their incorporation into commercial cultivars is a priority.

Interestingly, the current study also failed to confirm the previously reported resistance to *M. graminicola* in rice germplasm (Yik and Birchfield, 1979; Bridge *et al.*, 2005), where Bonnet 73 and LA 110 were considered resistant to a Louisiana isolate of *M. graminicola,* and Dumai, Germ and IR 20 were reported as resistant to an Indian isolate of the same nematode. These lines were susceptible to the Nepali isolate *M. graminicola* used in the current study. The contradictory results might be due to differences in the nematode isolates used, experimental conditions and/or methods of inoculation and evaluation index used (RGS *vs* RF) to evaluate the resistance. A difference in cultivar-by-isolate interaction in rice and this nematode was observed (Pokharel *et al.*, 2005), which further underlines the possibility of existence of higher variability in isolates. However, development of virulent populations in *M. graminicola* over time, especially in south Asian countries, including Nepal, is likely to happen where rice is cultivated up to three times a year without proper crop rotation. Such virulent populations might have developed by selection pressure or breaking down the resistant genes since this species has both sexual and asexual reproduction, and pathogens with a mixed reproduction system have the greatest ability to break down resistance genes (McDonald and Linde, 2002). Several other studies have also failed to identify rice germplasm with resistance to this nematode (Chunram, 1981; Rao *et al.*, 1986; Prasad *et al.*, 1986; Taya and Dabur, 2004). Despite the reported resistance to *M. graminicola* in rice germplasm/wild rice, breeding rice cultivars for resistance to this nematode has remained elusive (Plowright *et al.*, 1999). Soriano *et al.* (1999) reported that some accessions of *Oryza longistaminata* A. Chev. *et* Roehr. and *O. glaberrima* Steud., 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. glaberrima* into cultivated *O. sativa* germplasm were not encouraging. Although *O. glaberrima* was highly resistant 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). But several back crosses may transfer inferior productivity characters into the new cultivar. 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 twenty wild species (Bonman *et al.*, 1992).

Generally, higher RGS ratings and RF values were observed when a germplasm selected in initial tests was re-evaluated. The contradictory results obtained may be due to the difference in environmental conditions (the season in which 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 difference in season. Environmental factors, including light intensity and temperature, are known to affect 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 conferring resistant against *M. incognita, M. arenaria* and *M. javanica* in tomato (Ho *et al.*, 1992) is sensitive to heat and is not effective at temperature above 28 °C (Williamson, 1999). 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 levels of rice cultivars to *M. graminicola* vary under different water management systems.

Generally, higher RF values for *M. graminicola* were observed on rice than on wheat indicating higher reproduction of this nematode in rice. The higher reproduction of the nematode might be due to the genetic make-up of the plants and/or the available root biomasses for nematode growth and reproduction. Rice has a greater root-mass than wheat, thereby supporting higher nematode reproduction (Gaur and Sharma, 1999). Large-rooted plants will allow more nematode reproduction and tolerate more damage than small rooted plants despite the latter having fewer invasion sites for the nematode (Elkins *et al.*, 1979). However, Soomro and Hague

(1992) stressed the importance of the genetic make-up of plants in determining feeding behaviour and reproduction of the nematode. Results of the variety × isolate interaction in rice and wheat (Pokharel *et al.*, 2005) provided further support to the hypothesis that genetic make-up of plants plays the greater role in the reproduction of *M. graminicola*.

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