Incidence of Root-Knot Nematode (*Meloidogyne graminicola*) and Resulting Crop Losses in Paddy Rice in Northern India

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Abstract

Surveys of major rice growing districts in the state of Uttar Pradesh in Northern India were conducted for 3 consecutive years during 2013 to 2015 under a government-funded major research project to determine the frequency of occurrence and disease incidence of the rice root-knot nematode, *Meloidogyne graminicola*, in rice paddy fields. More than 800 paddy fields from 88 Tehsils (divisions within a district) in 18 major rice growing districts in Uttar Pradesh were surveyed, where *M. graminicola* was associated with root-knot disease in rice paddy fields based on morphological and molecular characterization of juveniles and adults. The highest frequency of disease in rice fields was observed in Aligarh (44.6%), followed by Muzaffarnagar, Shahjahanpur, and Kheri Lakhimpur (29.3, 28.0, and 27.4%, respectively). Maximum disease incidence was also recorded in Aligarh (44.6%), followed by Sultanpur, Mainpuri, and Muzaffarnagar (5.7, 5.2, and 4.5, respectively).

Gall index and egg mass index values (on a 0 to 10 scale) were highest in Aligarh (3.5 and 2.1, respectively), followed by Muzaffarnagar (2.6 and 2.0) and Mainpuri (2.3 and 1.8). The average soil population of *M. graminicola* was highest in Aligarh ($3,851 \pm 297$ second-stage juveniles [J2]/kg of soil), followed by Muzaffarnagar ($2,855 \pm 602$ J2/kg of soil), whereas the lowest population was recorded in Barabanki (695 ± 400 J2/kg of soil) at the time of harvesting. Relative yield losses were also determined, and the highest yield loss attributed to *M. graminicola* infestation was recorded in Aligarh (47%). The yield loss was linearly correlated with the soil population density of *M. graminicola* and disease incidence.

Keywords: paddy rice, survey, soil population, frequency of occurrence, yield loss

Rice is one of the most important food crops in the world and forms the staple food for more than half of the global population (McCouch et al. 2016). More than 90% of the world's rice is grown and consumed in Asia. In India, rice is extensively grown and occupies about 23.3% of the gross cropped area of the country, covering about 43 million hectares of land (Food and Agriculture Organization of the United Nations 2018). Rice contributes 43% of the total food grain production and 46% of the total cereal production in India. In 2016, India produced 15.88 million tons of rice, with an average productivity of 3,695 kg/ha, which is 25.5% lower than the global average rice productivity of 4,637 kg/ha (Food and Agriculture Organization of the United Nations 2018). Various biotic and abiotic factors contribute to the lower productivity of rice in India. Among the biotic factors, nematode infestation is considered an important factor responsible for up to 72% of yield loss (Khan et al. 2014; Prasad 2011). Economically important plant nematodes in rice are *Aphelenchoides besseyi*, *Ditylenchus angustus*, *Hirshmanniella oryzae*, *Meloidogyne graminicola*, *Heterodera indicus*, *Hoplolaimus* spp., and *Pratylenchus penetrans* (Haque et al. 2018; Prasad et al. 2010).

Among plant nematodes, the root-knot nematode, *M. graminicola*, has become an emerging problem in paddy cultivation (Haque et al. 2018; Jones et al. 2013). This nematode is highly damaging to upland, lowland, and deep water rice and causes serious economic

losses to rice grown in the nursery as well as in the field (Haque et al. 2018). The nematode is widely distributed in Southeast and South Asia (Bridge et al. 1990). The rice root-knot nematode has been recorded in some states of India, such as Jammu and Kashmir (Singh et al. 2007), Kerala (Sheela et al. 2005), Karnataka (Prasad et al. 2010), Haryana, Himachal Pradesh, Punjab (Dabur and Jain 2005; Jain et al. 2012), and Uttar Pradesh (Hassan et al. 2004; Khan et al. 2010), but its distribution and effects on yield have not yet been determined.

Infection of rice with *M. graminicola* results in growth reduction, reduced tillering, chlorosis of young plants, and production of unfilled spikelets at maturity (Babatola 1984). On roots, the nematode incites the formation of hook, spiral, or horseshoe-shaped terminal galls (Hunt and Handoo 2009). Transformation of roots into galls leads to impairment of water and mineral uptake. As a result, seedlings in the nursery or plants in the field show yellowish leaves, sparse tillering, and stunted growth (Khan and Anwer 2011). *M. graminicola* infestation in paddy fields may reduce yield by 15 to 30% (Khan et al. 2014; Prasad et al. 1986; Prot et al. 1994). Plowright and Bridge (1990) reported that *M. graminicola* infestation may suppress rice yield by 20 to 80% depending on the population density of the nematode. A preplant population of 1,500 juveniles of *M. graminicola*/kg of soil may reduce the rice yield by 27 to 35% (Haque et al. 2018; Khan et al. 2014).

Because of extensive and continuous paddy rice cultivation especially under irrigation, the rice root-knot nematode has emerged as a serious nematode pest in Northern India, especially in the state of Uttar Pradesh (Haque et al. 2018). Despite the widespread occurrence of *M. graminicola*, adequate information on its distribution and the extent of crop damage in India is not available. As a result, management strategies against this nematode have not been implemented properly. Hence, systematic and extensive surveys of all tehsils at the block level (subdivisions within a tehsil) in 18 major rice growing districts spread over a total area of 71,901 km² in Uttar Pradesh were conducted. The surveys were undertaken for 3 years (2013 to 2015) from July to October, and the incidence and population densities of *M. graminicola* were determined. Relative yield loss (RYL) in paddy rice as a result of this nematode was also assessed.

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Materials and Methods

Sample collection. Paddy fields in 18 major rice growing districts, including Aligarh, Allahabad, Azamgarh, Bahraich, Ballia, Barabanki, Deoria, Fatehpur, Ghaziabad, Hardoi, Kheri (Lakhimpur), Mainpuri, Muzaffarnagar, Pilibhit, Rampur, Saharanpur, Shahjahanpur, and Sultanpur, were surveyed. Ten paddy fields were surveyed in each block of various tehsils in each of the 18 districts. Ten soil samples each of soil and plants were collected from one field in a stratified random manner. The surveys were conducted at different stages of the crop, including the nursery stage (2 to 3 weeks after transplanting), the maturing stage (grain filling), and at harvest. The root and soil samples collected from one field (10 fields per block) were used to determine the soil population of *M. graminicola*, frequency of disease occurrence, and disease incidence.

Rhizosphere soil from five rice plants was collected in poly bags and brought to the laboratory (200 g per plant, 1,000 g per field). The composite soil sample (1 kg) was mixed in 8 liters of water and processed using Cobb's sieving and decanting method (using 10-, 35-, and 500-mesh sieves with 2,000-, 500-, and 26- μ m pores, respectively). The suspension caught on the 500-mesh sieve was incubated on a Baermann funnel for 12 h to isolate and count the *M. graminicola* second-stage juveniles (J2s) present in 1 kg of soil (Southey 1986). A precaution of continuously shaking the sieve set and decanting a smaller amount of suspension was taken to prevent clogging of the 500-mesh sieve pores. For counting, five 1-ml subsamples were taken from each sample and counted. The average of the five counts was used for further analysis.

Disease occurrence and root-knot incidence. The frequency of occurrence of root-knot of rice in each block of a tehsil of a district and the disease incidence in a field were calculated using the following formulas:

Frequency of occurrence (%)

$$= \frac{\text{Fields showing root} \\ \text{– knot symptoms on rice plants}}{\text{Total rice fields inspected}} \times 100$$

Disease incidence (%)

$$= \frac{\text{Number of plants infected with root} \\ \text{– knot nematodes in a field}}{\text{Total number of plants examined}} \times 100$$

Root-knot symptoms. Above-ground symptoms such as chlorotic leaves, unthrifty foliage, and stunted plants were recorded during the surveying of the paddy fields. Thereafter, the plants were carefully uprooted to observe root galls, the characteristic symptom of nematode attack. Values for the gall index and the egg mass index per root system were determined in the laboratory. For frequency of occurrence, a field was considered infected when plants growing in the field exhibited characteristic galls on roots. Similarly, for the field disease incidence, a plant was considered infected when it showed root galls.

Root-knot and egg mass indices. The root samples were stained in acid fuchsin and lactophenol to observe galls and egg masses (Byrd et al. 1983). *M. graminicola* gall and egg mass index values were determined according to the standard evaluation scale for the rice root-knot index (Bridge et al. 2005) as follows: 0, no gall or egg mass on the root system; 1, 10% of roots with galls or egg mass; 2, 20% of roots with galls or egg mass; 3, 30% of roots with galls or egg mass; 4, 40% of roots with galls or egg mass; 5, 50% of roots with galls or egg mass; 6, 60% of roots with galls or egg mass; 7, 70% of roots with galls or egg mass; 8, 80% of roots with galls or egg mass; 9, 90% of roots with galls or egg mass; and 10, all roots with galls or egg mass.

Yield loss assessment. The yield loss assessment was conducted with the difference between the attainable and the actual yield (Zadoks 1985). The functional relationships between production situation, attainable yield, and actual yield were calculated using the following formula:

$$RYL = 100 (Y_a - Y) / Y_a$$

where Y_a is the attainable yield as per specification of the cultivar and Y is the yield actually obtained (Zadoks 1985).

Identification of *M. graminicola*. The identity of *M. graminicola* was determined on the basis of morphological characteristics of J2s, perineal patterns of mature females, and molecular characteristics.

Morphology of juveniles. Morphological characteristics of J2s extracted from soil samples as well as those obtained from egg masses excised from the infected rice roots were studied (Hussey and Barker 1973). The juveniles were killed and fixed by adding an equal volume of boiling and double-strength triethanolamine formalin fixative (TAF) to the nematode suspension in water (Courtney et al. 1955). Temporary mounts of J2s were prepared in TAF on glass microscope slides. Morphometric measurements were recorded using an ocular micrometer at 400 $\times$ magnification.

Perineal pattern. Adult females were isolated from galled root. The females were placed in 45% lactic acid on a glass slide, and the perineal pattern was cut from the posterior end of the female. The section was cleaned and trimmed. Thereafter, the perineal pattern was transferred to another slide, with the outer surface side up, and mounted in glycerin with a glass coverslip for microscopic observation.

Molecular identification. Genomic DNA from the juveniles was extracted by maceration using proteinase K, as described previously (Subbotin et al. 2001). The genomic DNA of the nematode was used for the amplification of region between 18S and 26S using sense 18S (5'-TGATTACGTCCCTGCCCTTT-3') and antisense 26S (5'-TTTCACTCGCCGTTACTAAGG-3') primers (Vrain et al. 1992) in a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad). The reaction mixture contained 1 U of Dr. Max DNA polymerase (Doctor Protein Inc.), 200 nM of dNTP mixture, 1.5 mM of $MgCl_2$, and 100 ng of template DNA. The amplification conditions were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. An aliquot of the amplified product was electrophoresed on 1% agarose. The remaining product was purified by column purification using the QIAquick PCR Purification Kit per the manufacturer's instructions. The purified PCR product was subjected to Sanger sequencing with the BigDye Terminator version 3.1 sequencing kit and a 3730xl automated sequencer (Applied Biosystems). Both strands of the amplified DNA were sequenced at the Macrogen sequencing facility in Seoul, Korea.

Statistical analysis. Surveys were conducted during 3 years (2013 to 2015) to collect the root and soil samples from all of the blocks of a district. In each block, 10 paddy fields were randomly selected for taking observations, and five soil and root samples were randomly collected from each field. The data obtained from five samples in a field were pooled to calculate the mean value. Hence, there were five replicates in a block, which were analyzed for the SD, SEM, and coefficient of variance (CV%) within a district using the R software Agricolae package (De Mendiburu 2019). Frequency of root-knot disease occurrence, disease severity, gall index, egg mass index, and *M. graminicola* soil population were determined from samples collected from nurseries and fields. Regression analysis of soil population versus yield, root-knot index versus yield, and soil population versus root-knot index was also performed using Microsoft Excel 2016.

For nematode body measurements, the data on 10 observations were pooled to calculate the mean value of the selected morphometric characteristics of *M. graminicola* J2s isolated from the 18 districts and analyzed for the SEM, SD, CV%, and least significant difference ($P \leq 0.05$). Duncan's multiple range test was used to distinguish the significant variation among the juveniles.

Results

Identity of rice root-knot nematode, *M. graminicola*. The adult females were pear shaped (globular to oval), and the perineal patterns corresponded with those described for *M. graminicola*. The perineal pattern was dorsoventrally ovoid. The dorsal arch was high and squarish with four semicircular whorls on each corner of the vulvar slit (Fig. 1). The tail arch was generally high and squarish, and the

tail terminal area was flat with shallow transverse striae. Lateral fields were not clearly visible. The striae were generally smooth and continuous around the entire pattern.

The J2s were short (0.3 to 0.5 mm) with a weak cephalic framework. The esophageal gland lobe overlapped the intestine ventrally. The tail tip tapered to a long, fine point with a long hyaline region. Morphometric measurements of J2s varied among the isolates collected from 18 districts of Uttar Pradesh (Table 1). Among all isolates, the juvenile body length ranged from 408 to 480 μ m, and this variation was significantly different ($P \leq 0.05$). However, variations in stylet length and a value (body length/maximum body width), b value (length/esophagus length), and c value (length/tail to terminus) were not significant (Table 1).

All isolates used in this study produced the same size amplicon with the primers. The 5.8s rDNA-internal transcribed spacer region gene sequences were subjected to a similarity search using BLAST to identify and download the similar sequences from the NCBI

database. The gene sequences obtained in this study were submitted to the GenBank Nucleotide database under accession numbers MH265215 and MH265945 (*M. graminicola* AMUMG-1 and AMUMG-2). Species were identified by a simple comparison with available sequences of known *M. graminicola*. Phylogenetic positions using the unweighted pair group method with the arithmetic mean method in MEGA-6 software in relation to other related organisms are shown in Figure 2. AMUMG-1 grouped with the isolates from Delhi, whereas AMUMG-2 grouped with the isolate from Tuku (Fig. 2).

Gall shape and position of egg masses. Galls observed on the rice roots collected during the surveys resembled typical *M. graminicola* symptoms. The galls were invariably terminal on the lateral roots and sharply curved, in almost a ring-like spiral or a horseshoe shape. Unlike other *Meloidogyne* species, the egg masses incited by *M. graminicola* were not formed on the root surface (gall). The egg masses were embedded within the galled tissue and

Fig. 1. Root system of paddy rice infested with *Meloidogyne graminicola*. A, Seedling. B, Mature plant. C, Perineal pattern.

Table 1. Morphometrics in selected characters of second-stage juveniles of *Meloidogyne graminicola* of the 18 districts of Uttar Pradesh²

District	Body length	Stylet length	a value	b value	c value
Aligarh	473.0 (466–479) $\pm$ 4.3 ab	11.6 (10.5–12.5) $\pm$ 0.8 a	28.9 (27.5–36.3) $\pm$ 6.2 ab	3.0 (2.9–3.3) $\pm$ 0.18 def	6.4 (6.1–6.6) $\pm$ 0.2 a
Allahabad	454.6 (441–460) $\pm$ 7.1 ef	10.5 (9.7–12.1) $\pm$ 1.0 b	29.6 (28.2–33.7) $\pm$ 2.8 ab	3.7 (2.7–4.3) $\pm$ 0.55 a	6.4 (5.7–6.7) $\pm$ 0.4 a
Azamgarh	435.0 (425–452) $\pm$ 9.1 klm	11.1 (9.5–11.4) $\pm$ 1.1 ab	30.0 (28.2–32.5) $\pm$ 1.7 ab	3.3 (2.7–4.5) $\pm$ 0.63 cd	6.3 (5.9–6.8) $\pm$ 0.3 a
Bahraich	459.4 (455–470) $\pm$ 5.6 bc	11.8 (11.5–12.6) $\pm$ 0.7 a	29.7 (26.8–34.4) $\pm$ 3.6 ab	2.9 (2.4–3.4) $\pm$ 0.36 def	6.3 (5.8–6.8) $\pm$ 0.3 a
Ballia	434.6 (430–440) $\pm$ 3.8 klm	11.0 (10.0–12.2) $\pm$ 0.9 ab	31.3 (27.3–37.2) $\pm$ 4.7 ab	2.7 (2.2–3.5) $\pm$ 0.43 f	6.4 (6.0–7.5) $\pm$ 0.6 a
Barabanki	462.8 (455–478) $\pm$ 8.5b	11.3 (9.8–12.5) $\pm$ 1.0 ab	29.1 (26.6–33.4) $\pm$ 2.9 ab	3.3 (2.8–4.2) $\pm$ 0.47 bc	6.3 (6.1–7.1) $\pm$ 0.4 a
Deoria	441.2 (433–450) $\pm$ 6.8 hij	11.4 (9.5–12.5) $\pm$ 1.4 a	28.4 (28.5–29.8) $\pm$ 1.1 ab	3.0 (2.4–3.8) $\pm$ 0.48 def	6.4 (5.9–7.1) $\pm$ 0.5 a
Fatehpur	439.0 (418–452) $\pm$ 12.0 ijk	11.3 (9.8–12.9) $\pm$ 1.3 a	30.2 (27.1–35.8) $\pm$ 3.5 ab	2.9 (2.1–3.7) $\pm$ 0.65 ef	6.4 (6.0–7.0) $\pm$ 0.4 a
Ghaziabad	450.8 (441–458) $\pm$ 6.0 fg	11.2 (9.9–12.2) $\pm$ 0.9 ab	27.9 (27.4–31.2) $\pm$ 1.8 b	3.3 (2.4–4.1) $\pm$ 0.67 cde	6.4 (6.1–6.9) $\pm$ 0.3 a
Hardoi	427.2 (420–433) $\pm$ 4.4 no	11.0 (9.5–12.5) $\pm$ 1.1 ab	29.3 (27.2–34.7) $\pm$ 4.4 ab	3.5 (2.8–4.5) $\pm$ 0.65 ab	6.4 (6.1–7.0) $\pm$ 0.3 a
Kheri (Lakhimpur)	437.8 (428–455) $\pm$ 10.2 jkl	11.8 (10.2–12.6) $\pm$ 0.9 a	29.8 (27.3–31.8) $\pm$ 1.7 ab	3.0 (2.2–3.9) $\pm$ 0.65 def	6.3 (5.6–6.8) $\pm$ 0.5 a
Mainpuri	444.4 (430–452) $\pm$ 7.9 hi	11.3 (9.5–12.5) $\pm$ 1.2 ab	29.2 (27.7–32.8) $\pm$ 2.2 ab	3.1 (2.3–4.1) $\pm$ 0.76 def	6.4 (6.0–7.0) $\pm$ 0.4 a
Muzaffarnagar	476.0 (472–480) $\pm$ 2.8 a	11.6 (9.6–13.5) $\pm$ 1.3 a	29.2 (28.6–31.8) $\pm$ 1.4 ab	3.3 (2.8–4.0) $\pm$ 0.40 abc	6.4 (6.2–6.9) $\pm$ 0.3 a
Pilibhit	457.4 (448–465) $\pm$ 5.7 cd	11.7 (9.9–12.8) $\pm$ 1.3 a	31.4 (27.1–38.8) $\pm$ 5.1 a	3.1 (2.5–3.7) $\pm$ 0.44 cde	6.4 (5.8–7.0) $\pm$ 0.4 a
Rampur	446.4 (435–455) $\pm$ 8.0 gh	11.4 (9.6–12.7) $\pm$ 1.2 a	28.9 (27.0–31.8) $\pm$ 1.6 ab	3.1 (2.5–3.8) $\pm$ 0.48 cdef	6.3 (6.1–6.8) $\pm$ 0.2 a
Saharanpur	474.0 (468–478) $\pm$ 3.9 ab	11.5 (9.5–13.5) $\pm$ 1.4 a	30.3 (27.0–35.2) $\pm$ 3.3 ab	2.8 (2.1–3.5) $\pm$ 0.48 f	6.4 (6.2–7.0) $\pm$ 0.3 a
Shahjahanpur	432.6 (425–441) $\pm$ 5.7 lmn	11.6 (10.0–12.5) $\pm$ 1.0 a	31.1 (26.8–37.8) $\pm$ 4.1 ab	3.0 (2.4–3.7) $\pm$ 0.44 def	6.3 (6.0–6.9) $\pm$ 0.3 a
Sultanpur	425.2 (408–441) $\pm$ 10.5 o	11.6 (9.5–14.1) $\pm$ 1.6 a	29.8 (27.6–33.0) $\pm$ 2.2 ab	2.8 (2.1–4.3) $\pm$ 0.79 f	6.4 (6.1–7.0) $\pm$ 0.3 a
SEM	2.094	0.303	1.229	0.150	0.102
CV%	1.042	5.968	9.233	10.812	3.567
LSD ($P \leq 0.05$)	5.90	0.85	3.46	0.42	0.29

² Each value is the mean (range) $\pm$ SD of 10 observations. Values within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. a value = body length/maximum body width, b value = length/esophagus length, c value = length/tail to terminus, CV% = coefficient of variance, and LSD = least significant difference.

generally became visible upon tearing the galls (Fig. 1; Yik and Birchfield 1979).

Frequency of root-knot disease occurrence. Nursery. The frequency of rice root-knot disease occurrence caused by *M. graminicola* in the nursery among the 18 districts of Uttar Pradesh varied from 4.5 to 23.2% (Table 2). District-wise, the higher frequencies of occurrence were recorded in Aligarh (23.2%) and Shahjahanpur (16.0%), and the lowest was recorded in Allahabad (4.5%; Table 2).

Field. The frequency of root-knot nematode occurrence in the rice fields varied from 7.5 to 44.6% (Table 3). The highest frequency was recorded in Aligarh (44.6%), with a variation of 17 (Ghabana Tehsil) to 59% (Khair Tehsil; Fig. 3).

Fig. 2. A molecular phylogenetic analysis of *Meloidogyne graminicola* based on the internal transcribed spacer sequences by the maximum likelihood method using a general time-reversible model.

Disease incidence in the nursery and field. The overall rice root-knot disease incidence surveyed in 18 districts of Uttar Pradesh varied from 12.6 to 36.2% (Table 2). The highest nursery disease incidence was in Muzaffarnagar (36.2%) and the lowest was in Azamgarh (12.6%). In the rice fields, the highest disease incidence was in Sultanpur (51.0%) and the lowest was in Azamgarh (20.3%; Table 3).

Gall and egg mass indices. Nursery. In the nursery beds, the gall index of *M. graminicola* varied from 1.7 to 4.9 (on a 0 to 10 scale), being highest in Aligarh (4.9) and lowest in Barabanki (1.7; Table 2). The egg mass index of *M. graminicola* in the nursery varied from 0.4 to 1.4 (on a 0 to 10 scale; Table 2).

Field. In the paddy fields, the gall index varied from 1.7 to 7.0 (on a 0 to 10 scale; Table 3). The highest galling in rice roots was recorded in Aligarh (Table 3). The egg mass index on rice plants collected from the fields varied from 1.5 to 4.4 (on a 0 to 10 scale; Table 3). The highest egg mass index was recorded in Aligarh and the lowest was recorded in Fatehpur and Bahraich (Table 3).

***M. graminicola* population in soil.** J2 levels ranged from 610 ± 320 J2/kg of soil (Barabanki) to 3,060 ± 237 J2/kg of soil (Aligarh; Table 2). Other plant parasitic nematodes recovered included *Helicotylenchus*, *Hoplolaimus*, *Tylenchorhynchus*, *Rotylenchus*, *Rotylenchulus*, *Ditylenchus*, *Trichodorus*, and *Hirschmanniella* species and some free-living nematodes (not identified).

Field. The soil population of *M. graminicola* was also recorded in the paddy fields in all 18 districts of Uttar Pradesh, with the highest population in Aligarh (3,851 ± 297 J2/kg of soil) and the lowest in Barabanki (695 ± 400 J2/kg of soil; Table 3). In addition to

Table 2. Frequency of occurrence, root-knot index, and soil population of *Meloidogyne graminicola* (MG) in nursery beds of paddy in the 18 districts of Uttar Pradesh²

District	Frequency of occurrence (%)	Disease severity	Gall index (0–10 scale)	Egg mass index (0–10 scale)	Soil population (MG J2/kg of soil)
Aligarh	23.2 ± 7.7 a	33.4 ± 4.8 abc	4.9 ± 0.20 a	1.3 ± 0.22 a	3,060 ± 237 a
Allahabad	7.7 ± 4.2 fg	20.9 ± 7.6 ef	2.0 ± 0.92 hi	0.7 ± 0.37 fg	677 ± 484 hi
Azamgarh	8.3 ± 4.1 fg	12.6 ± 5.0 g	2.3 ± 0.72 ghi	0.8 ± 0.41 ef	915 ± 468 fghi
Bahraich	8.8 ± 2.9 f	18.5 ± 3.9 fg	1.8 ± 0.55 f	0.4 ± 0.22 i	616 ± 195 i
Ballia	11.7 ± 2.7 cde	26.8 ± 4.8 de	2.1 ± 0.48 hi	0.7 ± 0.31 fg	924 ± 318 fghi
Barabanki	9.0 ± 2.4 ef	28.3 ± 4.7 cd	1.7 ± 0.67 f	0.7 ± 0.37 fg	610 ± 320 i
Deoria	11.6 ± 1.7 cde	28.4 ± 2.9 cd	2.9 ± 0.59 efg	1.2 ± 0.18 b	1,015 ± 187 defg
Fatehpur	5.8 ± 2.9 gh	19.3 ± 6.1 f	1.8 ± 0.70 f	0.6 ± 0.25 fgh	787 ± 363 ghi
Ghaziabad	9.3 ± 1.5 ef	26.3 ± 13.0 de	3.4 ± 1.47 cde	1.1 ± 0.45 bc	1,291 ± 501 cde
Hardoi	12.8 ± 2.9 cd	28.8 ± 3.1 cd	2.4 ± 0.63 ghi	1.2 ± 0.25 b	985 ± 136 efg
Kheri (Lakhimpur)	13.4 ± 4 bc	32.0 ± 2.3 abcd	3.5 ± 0.45 cde	1.4 ± 0.12 a	1,458 ± 197 c
Mainpuri	12.7 ± 2.6 cd	33.3 ± 4.1 abc	3.9 ± 0.31 bc	1.2 ± 0.39 b	1,980 ± 482 b
Muzaffarnagar	13.5 ± 3.7 bc	36.2 ± 4.6 a	4.3 ± 0.21 ab	1.1 ± 0.24 bc	2,298 ± 333 b
Pilibhit	14.0 ± 3.7 bc	27.0 ± 1.6 de	3.1 ± 0.53 ef	1.0 ± 0.25 d	1,336 ± 202 cd
Rampur	4.5 ± 2.8 h	21.3 ± 14.9 ef	2.2 ± 1.43 hi	0.8 ± 0.47 ef	846 ± 488 defg
Saharanpur	10.2 ± 2.5 def	26.4 ± 5.2 de	2.7 ± 0.77 fgh	1.1 ± 0.20 bc	1,361 ± 366 c
Shahjahanpur	16.0 ± 3.1 b	29.8 ± 3.4 bcd	3.2 ± 0.69 def	1.1 ± 0.20 bc	1,191 ± 216 cdef
Sultanpur	13.0 ± 7.8 cd	36.0 ± 3.4 ab	3.9 ± 1.07 bcd	1.3 ± 0.17 a	1,974 ± 775 b
SEM	0.136	0.055	64.757	0.559	1.247
CV%	10.48	10.433	11.093	10.953	10.338
LSD ($P \leq 0.05$)	1.56	3.48	0.38	0.15	179.98
ANOVA					
District					
df	17	17	17	17	17
Sum of squares	1,517.6	3,479	73.60	13.658	35,990,917
Mean squares	89.27	204.66	4.330	0.8034	2,117,113
F value	58.15	26.78	47.63	54.94	103.9
Pr(>F)	<2e-16*	<2e-16*	<2e-16*	<2e-16*	<2e-16*
Residuals					
df	72	72	72	72	72
Sum of squares	110.5	550	6.55	1.053	1,467,485
Mean squares	1.54	7.64	0.091	0.0146	20,382

² Each value is the mean ± SD of all observations from the different blocks of a district. Values followed by different letters within the column are significantly different at $P \leq 0.05$ according to Duncan's multiple range test. * Values are significant at $P \leq 0.05$. J2 = second-stage juvenile, CV% = coefficient of variance, LSD = least significant difference, ANOVA = analysis of variance, df = degrees of freedom, and Pr = P-value for the effect of the classification variable on the response.

M. graminicola, other nematodes, especially *Hemicriconemoides*, *Hoplolaimus*, *Hemicylichophora*, *Tylenchorhynchus*, *Rotylenchus*, *Rotylenchulus*, *Diitylenchus*, *Trichodorus*, *Pratylenchus*, *Helicotylenchus*, and *Hirschmanniella* species and some free-living nematodes, were also recovered in the soil samples, and their cumulative populations were approximately 4 to 9% of the *M. graminicola* population.

Relative yield loss. The RYL attributable to root-knot disease caused by *M. graminicola* ranged from 7.0 to 46.7%. Besides the nematode attack, soil nutrition and other diseases may also contribute to the yield suppression. However, in view of the economic value of rice, growers generally give recommended inputs of fertilizers and pesticides to the fields. Hence, the lower yields may be conceivably attributed to *M. graminicola*, as any visible symptom attributable to other diseases was not observed. The maximum RYL was recorded in Aligarh and the lowest in Barabanki (Table 3).

Discussion

On the basis of morphology and molecular data, the identity of a root-knot nematode species causing galling on rice plants collected from nurseries and paddy fields in 18 districts of Uttar Pradesh was confirmed as *M. graminicola*. This species was previously reported as a causal agent of root-knot of rice in scattered areas in India (Dabur et al. 2004; Khan et al. 2010; Singh et al. 2007). This study on the occurrence of *M. graminicola* on rice from 18 districts of Uttar Pradesh is the first comprehensive report of rice infestation with *M. graminicola*. This report was made from systematic surveys covering a large area spread over 71,901 km² in Northern India conducted during 2013 to 2015 under a government-funded research project.

The nematode-infested nurseries showed stunted growth and discernable leaf yellowing, which are characteristic symptoms of *M. graminicola* damage at the seedling stage (Luc et al. 2005). The nematode causes a characteristic hook shape or spiral terminal galls on rice roots (Ahmad and Khan 2018; Dabur et al. 2004; Sheela et al. 2005). Galling severity varied considerably among the districts surveyed. There was a direct correlation between disease severity and J2 soil population densities of *M. graminicola* at harvest as revealed by regression analysis (Fig. 4). As a result, the areas/fields having a higher nematode population exhibited severe galling (higher disease severity). For example, in Aligarh, Muzaffarnagar, and Mainpuri, the soil population of *M. graminicola* at harvesting time was as high as 3,851, 2,855, and 2,650 J2/kg of soil and the RYLs were 46.7, 35.7, and 34.9%, respectively. Poudyal et al. (2005) reported that an initial population of 1,000 and 10,000 *M. graminicola* J2/kg of soil may reduce the rice yield by 31 and 97%, respectively. The yield loss reported above at the given population level is much greater than our observations. In fact, Poudyal et al. (2005) measured the nematode population at the planting stage, which would have increased during the crop season. However, in our study, the population was measured at the harvesting stage and would have been considerably lower at planting time. Other experiments have shown that 4,000 *M. graminicola* juveniles per plant may cause up to 72% crop loss (Bridge and Page 1982), whereas 1,500 J2/kg of soil reduces the yield by 27 to 32% (Haque et al. 2018). In this study, a strong negative correlation was recorded for yield versus soil population at harvest ($R^2 = 0.869$) and for yield versus root-knot index ($R^2 = 0.937$) and a positive correlation was recorded for soil population at harvest versus root-knot index ($R^2 = 0.918$; Fig. 4).

Table 3. Frequency of occurrence, root-knot index, and soil population of *Meloidogyne graminicola* (MG) in paddy fields at maturity stage (3 months) in the 18 districts of Uttar Pradesh^z

District	Frequency of occurrence (%)	Disease severity	Gall index (0–10 scale)	Egg mass index (0–10 scale)	Soil population (MG J2/kg of soil)	Yield (Q/ha)	Relative yield loss (%)
Aligarh	44.6 ± 15.0 a	47.9 ± 6.8 ab	7.0 ± 0.43 a	4.4 ± 0.37 a	3,851 ± 297 a	21.33 ± 0.30 c	46.68 ± 0.76 a
Allahabad	15.2 ± 6.9 fgh	33.4 ± 14.2 efg	2.0 ± 0.63 ghi	1.6 ± 0.48 ij	720 ± 605 hi	36.33 ± 2.34 a	9.18 ± 5.85 fg
Azamgarh	15.7 ± 7.6 fg	20.3 ± 7.9 h	2.4 ± 0.44 fghi	1.8 ± 0.33 hij	1,150 ± 585 fghi	35.18 ± 3.50 a	12.05 ± 8.75 ef
Bahraich	11.8 ± 4.3 ghi	26.5 ± 5.2 gh	1.8 ± 0.32 hi	1.5 ± 0.29 j	705 ± 243 i	36.59 ± 1.12 a	8.52 ± 2.80 i
Ballia	21.2 ± 7.4 de	33.3 ± 6.4 efg	2.5 ± 0.47 fgh	2.1 ± 0.41 ghij	1,175 ± 398 fghi	36.52 ± 2.46 a	8.70 ± 6.16 fg
Barabanki	17.8 ± 2.9 ef	34.5 ± 4.8 efg	1.7 ± 0.42 i	1.6 ± 0.37 j	695 ± 400 i	37.19 ± 0.67 a	7.03 ± 1.67 g
Deoria	23.4 ± 5.0 cd	35.0 ± 4.4 ef	3.0 ± 0.24 def	2.4 ± 0.25 efgh	1,122 ± 293 defg	31.40 ± 1.06 ab	21.50 ± 2.66 c
Fatehpur	10.3 ± 5.2 hi	28.0 ± 9.0 fgh	1.8 ± 0.32 hi	1.5 ± 0.26 j	980 ± 233 ghi	36.31 ± 2.91 ab	9.22 ± 7.29 fg
Ghaziabad	16.8 ± 4.1 efg	33.5 ± 17.4 efg	3.4 ± 0.41 de	2.9 ± 0.32 de	1,625 ± 454 cde	31.22 ± 4.71 ab	21.95 ± 11.77 c
Hardoi	25.4 ± 3.6 bcd	34.6 ± 3.8 efg	2.6 ± 0.87 efg	2.2 ± 0.65 fghi	1,220 ± 626 efgh	33.95 ± 2.27 a	15.13 ± 5.68 de
Kheri (Lakhimpur)	27.4 ± 5.2 bc	40.0 ± 2.0 bcde	3.8 ± 0.33 cd	3.0 ± 0.24 cdr	2,230 ± 170 c	32.70 ± 3.20 ab	18.25 ± 8.00 cd
Mainpuri	24.7 ± 10.0 bcd	46.0 ± 7.0 abc	4.5 ± 0.28 bc	3.6 ± 0.21 bc	2,650 ± 246 b	26.01 ± 3.43 bc	34.98 ± 8.59 b
Muzaffarnagar	29.3 ± 7.8 b	45.0 ± 6.9 abcd	5.2 ± 0.34 b	4.0 ± 0.25 ab	2,855 ± 602 b	25.74 ± 4.01 bc	35.65 ± 10.03 b
Pilibhit	24.3 ± 4.6 bcd	37.0 ± 3.7 de	3.4 ± 0.50 de	2.6 ± 0.25 efg	1,610 ± 749 cd	32.78 ± 1.64 ab	18.05 ± 4.10 cd
Rampur	7.5 ± 5.4 i	27.0 ± 19.0 fgh	2.8 ± 0.29 ef	2.0 ± 0.22 ghij	765 ± 417 ghi	35.15 ± 2.88 a	12.13 ± 7.20 ef
Saharanpur	15.0 ± 4.1 fg	33.8 ± 6.9 efg	2.8 ± 0.33 ef	2.8 ± 0.28 ef	1,750 ± 253 c	31.99 ± 1.95 ab	20.03 ± 4.87 cd
Shahjahanpur	28.0 ± 2.8 bc	39.0 ± 3.7 cde	3.4 ± 0.81 de	2.8 ± 0.54 ef	1,200 ± 730 cdef	32.39 ± 1.83 ab	19.03 ± 4.59 cd
Sultanpur	21.0 ± 16.3 de	51.0 ± 11.6 a	4.6 ± 0.34 bc	3.5 ± 0.31 bcd	2,425 ± 457 b	25.72 ± 2.79 bc	35.70 ± 6.99 b
SEM	1.027	1.660	0.158	0.120	80.627	1.493	0.967
CV%	10.958	10.348	10.816	10.477	11.130	10.385	11.073
LSD ($P \leq 0.05$)	2.86	4.64	0.44	0.43	224.06	4.25	2.7
ANOVA							
District							
df	17	17	17	17	17	17	17
Sum of squares	6,369	5,427	159.22	66.25	57,214,449	1,799.0	11,241
Mean squares	374.6	319.3	9.366	3.897	3,365,556	105.82	661.2
F value	72.7	23.48	77.06	54.81	106.6	9.278	138.1
Pr(>F)	<2e-16*	<2e-16*	<2e-16*	<2e-16*	<2e-16*	3.64e-162*	<2e-16*
Residuals							
df	72	72	72	72	72	72	72
Sum of squares	371	979	8.75	5.12	2,272,964	821.2	345
Mean squares	5.2	13.6	0.122	0.071	31,569	11.41	4.8

^z Each value is the mean ± SD of all observations from the different blocks of a district. Values followed by different letters within the column are significantly different at $P \leq 0.05$ according to Duncan's multiple range test. * Values are significant at $P \leq 0.05$. J2 = second-stage juvenile, Q/ha = quintal per hectare, CV% = coefficient of variance, LSD = least significant difference, ANOVA = analysis of variance, df = degrees of freedom, and Pr = P-value for the effect of the classification variable on the response.

During the surveys, an overall yield loss of 20% could be attributed to the nematode infestation. The highest yield loss (44.6%) compared with noninfested fields was recorded in Aligarh. Yield losses attributable to *M. graminicola* in upland rice have been estimated to be 16 to 32% (Haque et al. 2018) and up to 64% under severe infection (Prot et al. 1994). The nematode infestation in the nursery was considerably high in all of the districts. *M. graminicola* is one of the few plant nematodes that are equally destructive in the nursery as well as in the field (Haque et al. 2018), as also observed during this study. During the survey, villagers informed the investigators that some farmers in Aligarh, Muzaffarnagar, and Sultanpur plowed their rice fields after 4 to 5 weeks of nursery planting because of seedling growth stagnation in the nematode-infested fields, which apparently took place as a result of planting of the *M. graminicola*-infested nursery in the nematode-infested fields. Infestation in the nursery was apparently the result of the continuous raising of nursery plants in the same plot. Furthermore, close spacing of seedlings and the ideal soil conditions with adequate organic matter and moisture coupled with

numerous young feeder roots available for juvenile invasion and feeding were largely responsible for the development of galls on nursery roots during a short span of 4 to 5 weeks.

This study demonstrates that the occurrence and incidence of root-knot nematodes of rice was alarmingly high in rice nurseries as well as in paddy fields in Aligarh, Lakhimpur, Muzaffarnagar, and Shahjahanpur. Farmers are generally not aware of the disease and do not use control measures to manage rice root-knot nematodes, particularly in nurseries. This has led to nematode-infested nursery stock of poor quality, which is a major source of nematode dissemination. Thus, it becomes essential to conduct regular surveys and extension work in rice growing areas to inspect rice nurseries for infestation with *M. graminicola* and advise farmers to execute appropriate management measures. Furthermore, the preparation of rice nurseries in fully or partially controlled environments, such as hydroponics, substrate culture, or in fields not having rice cultivation for at least the last 3 years, could prove to be very effective in preventing nematode infection in rice nurseries.

Fig. 3. District-wise distribution of frequency of occurrence and disease severity in the Aligarh, Allahabad, Azamgarh, Bahraich, Ballia, Barabanki, Sultanpur, Deoria, Fatehpur, Ghaziabad, Hardoi, Kheri Lakhimpur, Mainpuri, Muzaffarnagar, Pilibhit, Rampur, Saharanpur, and Shahjahanpur districts.

Fig. 4. Regression analysis between **A**, yield and *Meloidogyne graminicola* (MG) soil population, **B**, yield and root-knot index, and **C**, soil population and root-knot index of 18 districts of Uttar Pradesh. Q/ha = quintal per hectare, Y = regression equation, and R^2 = coefficient of regression.

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