

Morphological, Morphometric, and Molecular Characterization of Intraspecific Variations within Indian Populations of *Meloidogyne graminicola*

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Abstract: Fourteen populations of *Meloidogyne graminicola* were collected from different agroecological regions of India. Morphological and morphometrical comparisons were made for various nematode life stages. Three populations (Hisar, New Delhi, and Samastipur) were different from typical *M. graminicola* on the basis of the length of eggs; J2 length, α -value, hyaline tail portion; male length, distance up to excretory pore, spicule and gubernaculum lengths; female length and width, stylet length, distance up to excretory pore, EPST (distance of excretory pore from anterior end / stylet length [females]) ratio, and vulval length. Morphological and morphometrical comparison with closely related species *M. graminis*, *M. oryzae*, *M. salasi*, *M. triticoryzae*, and *M. lini* clustered these populations into two groups: Anand, Bhubaneswar, Hyderabad, Jammu, Jorhat, Kalyani, Kanpur, Ludhiana, Mandya, Palampur, Vellayani grouped with *M. graminicola*, *M. triticoryzae* and *M. salasi*; whereas, Hisar, New Delhi, Samastipur grouped with *M. oryzae* and *M. graminis*. Molecular phylogenetic analysis using internal transcribed spacer (ITS) suggested that in spite of morphological differences, these populations belonged to *M. graminicola*.

Key words: agglomerative hierarchical clustering, diversity, internal transcribed spacer, *Meloidogyne graminicola*, molecular characterization, morphology, morphometrics, multivariate analysis, phylogenetics, variation.

The rice root-knot nematode, *M. graminicola* (Golden and Birchfield, 1965) has emerged as a pest of global importance. In India, it was observed for the first time in 1969 in association with rice (Patnaik, 1969). Initially, it was confined to West Bengal, Odisha, Assam, and Kerala states, but of late, it has spread to Uttar Pradesh, Delhi, Haryana, Punjab, Himachal Pradesh, Jammu & Kashmir, Tamil Nadu, Karnataka, and Gujarat (Prasad et al., 1987; Jain et al., 2012). Infestations are particularly severe where two crops of rice are taken in a year, or where graminaceous weeds are abundant between two rice crops (Pankaj et al., 2010). Pockets of heavy infestation of rice nurseries and transplanted crop have been noticed in North Indian plains including Jammu (J&K), Punjab, Himachal Pradesh, Haryana, Delhi, and Uttar Pradesh (Gaur et al., 1996; Pankaj et al., 2006; Singh and Singh, 2009).

Considering the enormity of rice germplasm being cultivated in India under diverse agroecological conditions, it is logical to believe that the associated parasitic nematodes may also possess genetic variability at specific and/or intraspecific level. Accurate identification, characterization, and ecological variations (ecotypes) are important to understand the host–parasite relationships and implement appropriate management options, including the development of nematode-resistant rice cultivars. Here we sought out to determine the intraspecific variations within the Indian populations of *M. graminicola* isolates from different agroecological zones based on morphological and morphometric

characterization. Molecular phylogenetic analysis of some populations using the ITS marker was also attempted to substantiate the morphological and morphometrical analysis.

MATERIALS AND METHODS

Fourteen populations of *M. graminicola* (Table 1) were collected or procured from various parts of India during summer 2012, multiplied using single egg mass, and maintained on rice plant cvs. Basmati 370 or Pusa Basmati 1121 in a screen-house and a growth chamber during summer and winter, respectively.

Obtaining eggs and J2 of *M. graminicola*: Eggs were extracted from infected rice roots maintained in culture pots either by dissecting galled roots or by using the NaOCl method (Hussey and Barker, 1973) combined with blending the roots in a waring blender at 20-sec intervals for 3 min. J2 were obtained from egg suspension by the modified Baermann funnel technique (Schindler, 1961).

Killing, fixing, and preparing nematode mounts: The live nematodes were concentrated in about 2 ml water, killed, and fixed by adding an equal volume of boiling double-strength triethanolamine formalin (TAF) fixative (Courtney et al., 1955). Temporary mounts of J2 and males were prepared in a small drop of TAF on a glass slide using glass wool fibers to support the cover glass that was sealed with nail polish. For en face views or body sections, J2 and males of *M. graminicola* fixed in TAF were cleared to pure glycerol (Seinhorst, 1966, 1973); the cleared nematodes or males obtained from stained roots were processed further as per procedure described by Hooper (1970) and mounted in glycerin jelly. Rice roots infected with *M. graminicola* were washed thoroughly and stained with acid fuchsin (McBeth et al., 1941). Fully developed females of *M. graminicola* were dissected out from the stained roots and cut into two pieces. The anterior half was trimmed further to retain the head end and was mounted in a drop of plain lactophenol. The

Received for publication March 26, 2017.

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This paper was edited by Andrea M. Skantar.

TABLE 1. Details of the *Meloidogyne graminicola* populations collected/procured from different rice-growing states of India.

Sr. No.	Place	Code	State	Latitude/Longitude	Source
1	Anand	AND	Gujarat	22°32'04.8"N 72°58'16.4"E	Department of Nematology, Anand Agricultural University, Anand
2	Bhubaneswar	BHB	Odisha	20°15'53.4"N 85°48'41.2"E	Department of Nematology, Orissa University of Agriculture and Technology, Bhubaneswar
3.	Hisar	HSR	Haryana	29°05'30.9"N 75°55'43.2"E	Farmer's field near Hansi (District Hisar)
4	Hyderabad	HYD	Telangana	17°19'12.8"N 78°23'38.6"E	Directorate of Rice Research, Hyderabad
5	Jammu	JAM	Jammu & Kashmir	32°39'25.5"N 74°48'10.8"E	Sher-e-Kashmir University of Agriculture & Technology, Jammu
6	Jorhat	JOR	Assam	26°43'21.8"N 94°11'44.0"E	Department of Nematology, Assam Agricultural University, Jorhat
7	Kalyani	KAL	West Bengal	22°56'54.7"N 88°31'52.0"E	Department of Entomology, Bidhan Chandra Krishi Vishwa Vidyalaya, Kalyani
8	Kanpur	KAN	Uttar Pradesh	26°29'28.6"N 80°18'26.4"E	Department of Entomology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur
9	Ludhiana	LUD	Punjab	30°54'11.4"N 75°48'31.7"E	Department of Plant Pathology, Punjab Agricultural University, Ludhiana
10	Mandya	MAN	Karnataka	12°31'12.4"N 76°53'58.5"E	Department of Plant Pathology, University of Agricultural Sciences, Bengaluru
11	New Delhi	DEL	Union Territory	28°37'46.8"N 77°09'30.1"E	Indian Agricultural Research Institute, New Delhi
12	Palampur	PAL	Himachal Pradesh	32°06'31.0"N 76°32'38.9"E	Department of Entomology, Himachal Pradesh Krishi Vishwa Vidyalaya, Palampur
13	Samastipur	SAM	Bihar	25°59'00.6"N 85°40'44.5"E	Department of Nematology, Rajendra Agricultural University, Pusa, Samastipur
14	Vellayani	VEL	Kerala	08°25'46.2"N 76°59'17.8"E	Department of Entomology, Kerala Agricultural University, Vellayani

posterior half was used for preparing perineal patterns and mounted similarly (Taylor et al., 1955).

Measurements of nematodes: Most of the measurements were recorded using ocular micrometer at $\times 40$ and $\times 100$. Only for curved structures that could not be measured accurately with ocular micrometer, camera lucida method was used. The following measurements (with codes) were recorded in different stages of *M. graminicola*: L = total body length (μm), MBW = maximum body width (μm), STYL L = stylet length (μm), DPGO = distance from stylet knobs to opening of dorsal pharyngeal gland (μm), EXC PORE = distance from anterior end to excretory pore (μm), PHX-C = distance from anterior end up to cardia (μm), PHX-G = distance from anterior end up to gland overlap (μm), TL = tail length (μm), HYL TL = length of hyaline portion of tail (μm), ABW = anal body width (μm), SPL = length of spicules (μm), GUB = length of gubernaculum (μm), VUL L = vulval length (μm); IPD = interphasmidial distance (μm); AVD = distance between anus and vulva (μm). de Man's ratios i.e., *a*, *b*, *b'*, *c*, and *c'* were calculated (Siddiqi, 2000). Other ratios namely EPST = distance of excretory pore from anterior end / stylet length (females) and L:W = length and width ratio (eggs) were also employed.

Duncan's multiple range test (DMRT) was used to determine the differences between means of the characters at $P = 0.05$ level of significance.

DNA isolation, sequencing, and phylogenetic analysis: For the molecular phylogenetic analysis, the genomic DNA

was isolated from approximately 50 freshly isolated females using DNeasy Blood & Tissue Kit (Cat No.: 69504; Qiagen, Valencia, CA). The ITS region was amplified with Vrain's primers using Taq DNA polymerase (Cat No.: 201203; Qiagen) using recommended PCR reaction conditions (Vrain et al., 1992). The amplicons were gel purified, cloned into PCR cloning vector PGEM-T (Cat. No.: A3600; Promega, Madison, WI) and sent for sequencing. The sequences obtained were manually verified for quality, processed, and used for phylogenetic analysis using MEGA 6 (Tamura et al., 2013). The evolutionary history of Indian *M. graminicola* populations was inferred by using the maximum likelihood method based on the general time reversible model (Tamura et al., 2013), which was suggested as the best model to analyze the data by MEGA 6. The bootstrap consensus tree inferred from 1,000 replicates was used to represent the evolutionary history of the analyzed *Meloidogyne* spp. (Felsenstein, 1985). ITS sequences from the nematodes *Hirschmaniella mucronata* (EU722287) and *Globodera rostochiensis* (GQ294519) were used as outgroups (Kimura, 1980).

RESULTS

Eggs ($n = 20$): Eggs of Samastipur population were the largest (mean length 108.1 μm) and on par with New Delhi, Hyderabad, and Hisar populations, whereas the Vellayani population eggs were the smallest (84.1 μm). The widest eggs belonged to the Kalyani population

(47.7 μm), whereas the Ludhiana (38.9 μm), Jammu (39.7 μm), and Vellayani (39.9 μm) population eggs recorded the least width. Eggs of Kalyani population were more round (L:W = 1.853), whereas Ludhiana population eggs were more oblong (L:W = 2.583) (Fig. 1A,B). Coefficient of variation (CV) values of egg length and width were quite low indicating stability of the parameters within populations (Table 2).

Second-stage juveniles (J2) ($n = 10$ to 16): Three distinct groups of populations were clearly discernible on the basis of J2 length. Group 1 was comprised of seven populations: Bhubaneswar, Jammu, Kanpur, Ludhiana, Mandya, Palampur, and Vellayani, each with a total body length always below 500 μm . Group 2 consisted of four populations: Hisar, Kalyani, New Delhi, and Samastipur, where the total body length ranged between 418.7 and 598.2 μm . Group 3 included three populations: Anand, Jorhat, and Hyderabad and to some extent Kalyani, which represented mixture of two types of J2—both short and long (Fig. 1C). Because there was little variation in maximum body width, the populations showing maximum total body lengths of J2 also recorded maximum a -values e.g., Hisar (37.2), New Delhi (37.8), Kalyani (39.2), and Hyderabad (37) (Table 3).

The lip region was flat anteriorly, continuous with body, and weakly sclerotized in all populations. The mean values of stylet length varied between 10.4 and 11.8 μm ; J2 of Anand, Bhubaneswar, Hisar, and Hyderabad populations possessed longer stylets (11.4 to 11.8 μm), whereas that of Palampur (10.4 μm) population had relatively shorter stylet. J2 of Samastipur population were distinct in having dorsally curved conus. Stylet knobs were sloping rounded in all populations. DPGO was significantly more among J2 of Hyderabad, Kalyani, and Samastipur (4 μm each) populations than in rest of the populations. J2 of Hyderabad, New Delhi, and Samastipur populations had the largest PHX-C. b -value was lowest (5.5) and at par in J2 of Jammu, New Delhi, and Hyderabad populations. The populations possessing longest pharynx (Hyderabad, Jorhat, Jammu, Mandya, New Delhi, and Samastipur) up to gland overlap recorded the least b' -values (<3). The distance of excretory pore from the anterior end was maximum (84.0 to 89.2 μm) in J2 of New Delhi, Hyderabad, Samastipur, Hisar, Kalyani, and Jorhat populations.

Maximum tail length (85.7 to 90.4 μm) was observed in J2 of Hyderabad, Samastipur, New Delhi, and Kalyani populations. J2 of Hisar population had distinctly higher c -value (7.2), which is significantly more than all other populations (6.0 to 6.6). Hyaline tail length (27.9 μm) was also maximum in J2 of Hyderabad, Kalyani, Samastipur, and New Delhi populations. Tail shape and tail terminus were rounded, often slightly clavate, more so in Samastipur population. Jammu and Jorhat J2 possessed the maximum (11.1 μm) and minimum

(10.1 μm) anal body width, respectively; in other populations, it was not significantly different. A swollen pre-rectum was a common feature of almost all the populations. J2 of Hyderabad (8.7), Samastipur (8.4), Kalyani (8.2), New Delhi (8.1), and Jorhat (8) were at par as far as c' -value is concerned. The number of lateral lines was universally four in all the populations, outer crenated (Fig. 1D).

Males ($n = 3$ to 12): Males of 13 populations were studied (no males recovered from Mandya population). Males of Hisar (mean 1,738.4 μm) and Samastipur populations (1,615.2 μm) were significantly longer than rest of the populations; whereas those of Ludhiana (1,118.6 μm) and Anand (1,261.2 μm) populations were the shortest. Males of Hisar population were most slender with mean a -value of 54.5, and it was significantly higher than all the remaining populations except New Delhi (48.9). Ludhiana population recorded the least a -value of 38.5 which is indicative of thicker males, besides being shorter (Table 4).

Lip region was continuous with the body, nearly flattened anteriorly, or slightly offset by a constriction in all populations. Samastipur and Hisar males possessed the longest stylets with mean lengths of 19.4 and 19.3 μm , respectively. Stylet knobs were backwardly sloping or slightly set-off from shaft in all populations. A conspicuous constriction was observed at the junction of shaft and knobs in Ludhiana population. DPGO was maximum in males of Hisar (5 μm) and it was at par with those of Hyderabad, Samastipur, Vellayani, and New Delhi populations. PHX-G could not be recorded for four populations namely Anand, Jammu, Kalyani, and Vellayani; it was maximum in New Delhi population (mean 240 μm), and significantly more as compared with others. Maximum value (8.1) of b' was recorded for Hisar population, and it was at par with that of Kanpur and Samastipur populations. The distance from the anterior end to excretory pore was maximum (145.8 μm) in males of Hisar population and it was at par with that of Samastipur population (135.2 μm).

Longest tail (12.8 μm) was recorded in Hisar population, which was at par with that in Kalyani and Palampur populations. The shortest tail was possessed by males of Ludhiana (9.3 μm), but it was at par with other populations. The tail terminus was generally smooth, but in New Delhi and Hyderabad populations, it was bifid. Maximum c -value (170) was observed for males of Samastipur population which was statistically similar to New Delhi (154) and Kanpur (147.5). Least c -value of 108.9 was recorded for Kalyani population. Hisar population exhibited maximum (20 μm) ABW and showed nonsignificant differences with Bhubaneswar, Kalyani, Kanpur, and Palampur populations. Ludhiana (15.1 μm) recorded the least ABW. There was little variation in the c' -value, and it ranged between 0.54 and 0.69.

Length of spicules mostly corresponded to total body length. With 31.5 μm , spicules of New Delhi population

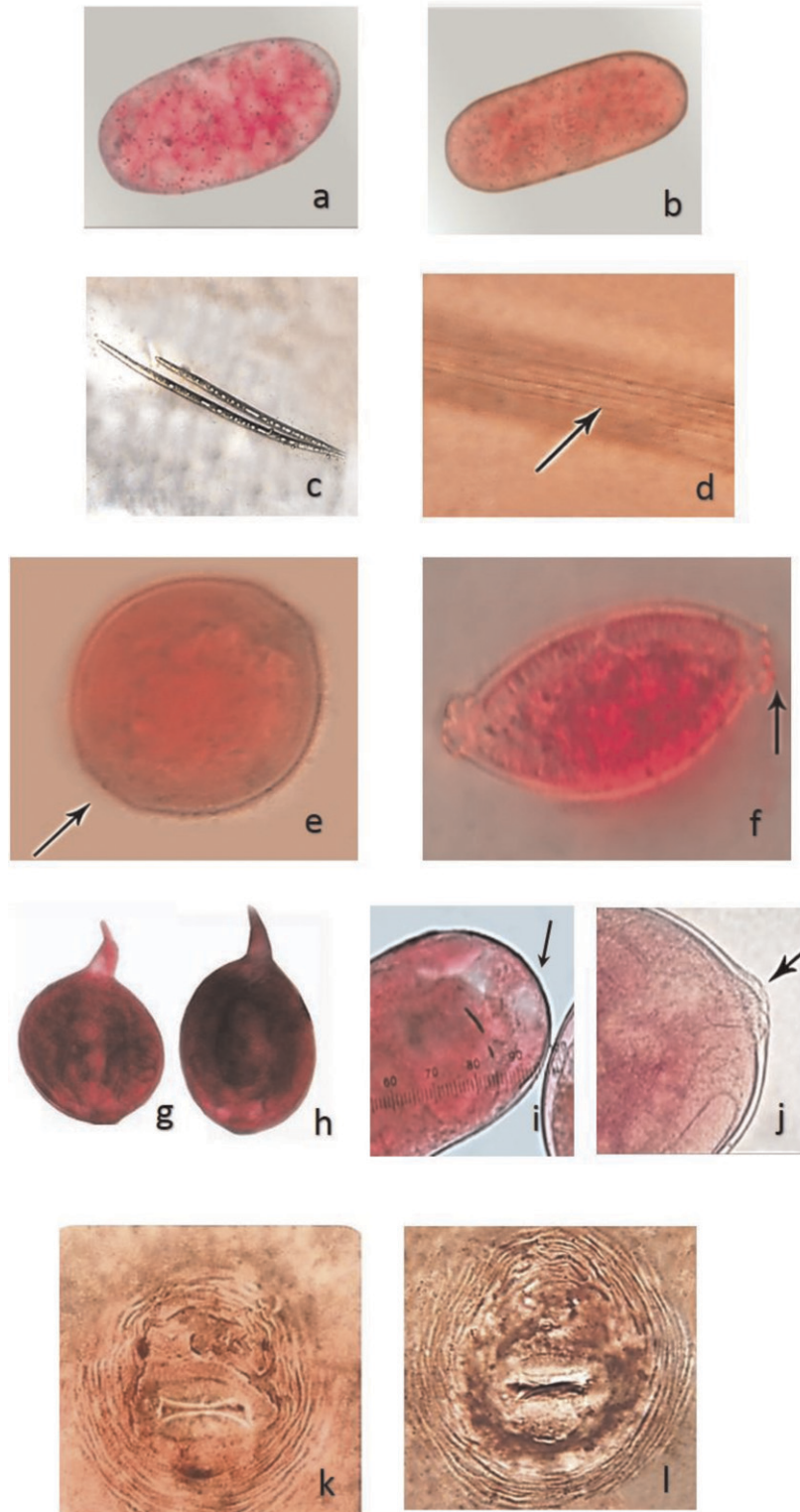


FIG. 1. Egg shapes: A. Round (Kalyani); B. Long (Ludhiana). J2 of *Meloidogyne graminicola* total body length: C. Long and short; D. Four lateral lines. Cross sections of male: E. Without lateral alae (New Delhi population); F. Six lateral lines with lateral alae. Female shapes: G. Round; H. Oval; I. Female without vulval protuberance; J. Female with vulval protuberance. Perineal pattern shapes: K. Oval; L. Round.

were the longest and at par with those of Hisar, Hyderabad, and Jorhat populations. A majority of the populations recorded spicular length between 27.4 and 29 μm . Maximum length of gubernaculum (7 μm) was

observed in Hisar and Samastipur males; however, there were no significant differences among populations, except Bhubaneswar that possessed the shortest gubernaculum (6.2 μm).

TABLE 2. Measurements of eggs of different populations of *Meloidogyne graminicola*.

Sr. No.	Populations	Length (μm)		Width (μm)		Ratio L:W
		(Range)	CV	(Range)	CV	
1	Anand	92.7de		42.3cde		2.191
		(84.0–98.0)	4.19	(39.0–46.0)	4.61	
2	Bhubaneswar	94.7de		42.8cd		2.212
		(86.0–115.0)	7.05	(40.0–48.0)	5.16	
3	Hisar	105.9ab		46.0ab		2.302
		(92.0–115.0)	5.61	(38.0–60.0)	11.74	
4	Hyderabad	106.6ab		45.4b		2.348
		(97.0–116.0)	5.00	(36.0–50.0)	6.21	
5	Jammu	92.8de		39.7gh		2.337
		(87.0–100.0)	4.15	(35.0–43.0)	5.67	
6	Jorhat	104.2b		41.0defgh		2.541
		(90.0–120.0)	7.17	(32.0–49.0)	10.37	
7	Kalyani	88.4f		47.7a		1.853
		(70.0–96.0)	6.62	(41.0–58.0)	9.84	
8	Kanpur	91.9e		41.3defg		2.225
		(85.0–97.0)	3.50	(38.0–45.0)	3.94	
9	Ludhiana	100.5c		38.9h		2.583
		(95.0–105.0)	3.04	(36.0–44.0)	5.56	
10	Mandya	92.1de		41.9def		2.198
		(83.0–105.0)	6.24	(34.0–48.0)	8.18	
11	New Delhi	106.7ab		45.3b		2.355
		(98.0–115.0)	4.22	(39.0–54.0)	9.56	
12	Palampur	95.4d		40.3efgh		2.367
		(87.0–107.0)	5.46	(37.0–44.0)	5.64	
13	Samastipur	108.1a		44.4bc		2.435
		(98.0–122.0)	6.24	(38.0–50.0)	7.25	
14	Vellayani	84.1g		39.9fgh		2.107
		(74.0–113.0)	9.58	(32.0–47.0)	8.79	
	SE(m)	0.6		0.25		
	CV %	9.5		9.8		

Means within a column followed by the same letter are not different ($P = 0.05$) according to Duncan's multiple range test. CV = coefficient of variation.

In a majority of the populations, the number of lateral lines ranged between 4 and 8 (Ludhiana, New Delhi, Hyderabad, Kanpur, Jammu, Samastipur, Palampur 4 to 8, Jorhat 4 to 6, Vellayani 4 to 7, Hisar 6 to 8, Anand 4 to 9, Bhubaneswar 4, Kalyani 6 and 7). New Delhi males did not show lateral alae, but lateral lines were present (Fig. 1E,F).

Females ($n = 10$ to 12): Female shapes were variable—globular and pear-shaped (oval) to elongated (Fig. 1G, H). Except New Delhi population, in all the other populations, 100% of females possessed a protuberance at the posterior end. In New Delhi population, only 64% females exhibited protuberance (Fig. 1I,J, Table 5).

Lip region was smooth, anteriorly flattened, not distinctly set off from neck in all populations. Females of New Delhi population were distinct from all others with mean body length of $697 \mu\text{m}$. Anand population recorded the least average female length of $476.9 \mu\text{m}$; in others, it was intermediate. Value of a was maximum (2) in Jammu and Vellayani populations which is indicative of relatively slender (oblong) females; whereas Anand and Palampur populations recorded least a -value, which may be suggestive of a predominantly roundish

shape. However, majority of the populations recorded a -value between 1.7 and 1.9.

Stylet length ($13.6 \mu\text{m}$) and DPGO ($5.9 \mu\text{m}$) were maximum in New Delhi population, and least in Vellayani population (9.3 and $4.0 \mu\text{m}$, respectively). Conus was dorsally curved in Jammu, Hisar, and Kalyani populations. Stylet knobs were rounded with posteriorly sloping anterior margins in all the populations.

Females of the New Delhi population were also distinctly different from the rest of the populations for having maximum distance ($40 \mu\text{m}$) from the anterior end up to excretory pore. Hisar and New Delhi populations exhibited the maximum EPST ratio with 2.94 and 2.93, respectively; least EPST ratio of 1.88 was recorded in Anand, followed by 1.96 in Kanpur population.

Vulval length was significantly more in the New Delhi ($30.8 \mu\text{m}$), Samastipur ($30.5 \mu\text{m}$), and Kalyani populations ($28.1 \mu\text{m}$); however, for a majority of the populations it ranged between 25.6 and $27.2 \mu\text{m}$. Bhubaneswar population recorded the least vulval length of $23.6 \mu\text{m}$ that was at par with Anand, Hyderabad, Jammu, Ludhiana, and Palampur populations. New Delhi ($19.5 \mu\text{m}$) population registered the maximum distance between vulva and anus, but it was at par with majority of the populations. Bhubaneswar population had the minimum distance ($14.8 \mu\text{m}$). Kalyani population with $16.7 \mu\text{m}$ distance between the phasmids ranked first but at par with Anand, Hisar, Jammu, Mandya, New Delhi, Samastipur, and Vellayani populations. Hyderabad ($12.8 \mu\text{m}$) and Palampur ($13.2 \mu\text{m}$) populations recorded minimum distance between phasmids along with Bhubaneswar, Jorhat, Kanpur, and Ludhiana populations. New Delhi, Palampur, and Anand populations were consistent in having round shape of the perineal pattern, whereas in Samastipur, Mandya, Kalyani, and Hisar populations, it was predominantly round. Vellayani population was distinct in possessing uniformly oval-shaped perineal pattern (Fig. 1K,L). No significant differences were observed among the populations as far as striation patterns are concerned.

Multivariate analysis: The discriminant analysis and hierarchical cluster-based analysis were not performed for each stage separately because the species diagnosis is a function of characteristics derived from all the stages i.e., egg, J2, male, and female. On the basis of $\text{Pr} > F (< 0.0001)$ (Table 6), 13 variables (Functions 1 to 13) were shortlisted. These are Egg L (F1), J2 L (F2), Female STYL L (F3), J2 STYL L (F4), Male DPGO (F5), Male b' (F6), J2 DPGO (F7), Female EXC PORE (F8), J2 b' (F9), J2 MBW (F10), Egg W (F11), Male ABW (F12), and J2 c' (F13). The genetic distance (Euclidean distances) in respect of Hisar population (C2) vis-à-vis other populations ranged between 166.122 and 283.229 v/s C4, between 298.6 and 446.178 v/s C3, and between 431.310 and 628.737 v/s C1 (Tables 7,8). Based on

TABLE 3. Measurements of 12 of different populations of *Meloidogyne graminicola*.

S. No.	Populations	n	L (µm)	MBW (µm)	a	STYL L (µm)	DPGO (µm)	PHX-C (µm)	b
1	Anand	15	484.6c (408.8-568.3)	14.9a (14.0-16.0)	32.4de (27.3-37.9)	11.4ab (11.0-12.0)	3.1bcd (3.0-4.0)	82.0cdef (71.0-92.0)	5.9abc (5.2-6.3)
2	Bhubaneswar	12	432.1d (358.9-478.6)	13.5d (12.0-15.0)	32.1e (27.1-37.4)	11.8a (11.0-12.5)	2.9d (2.9-3.0)	74.9fg (68.0-80.0)	5.8abc (5.1-6.4)
3	Hisar	14	531.9ab (488.5-568.3)	14.4abc (13.0-17.0)	37.2abc (29.3-42.9)	11.8a (11.0-12.0)	3.1bcd (2.0-4.0)	85.3cd (67.0-93.0)	6.3a (5.6-7.3)
4	Hyderabad	11	540.2ab (408.8-608.2)	14.6ab (14.0-15.0)	37.0abc (27.3-42.7)	11.6ab (10.0-12.0)	4.0a (3.0-4.5)	100.2a (70.0-121.0)	5.5cd (4.4-6.2)
5	Jammu	10	437.7d (398.8-458.6)	14.7ab (14.0-15.0)	29.8ef (26.6-32.8)	10.7c (10.0-11.0)	3.0cd (3.0-3.0)	89.0cb (70.0-110.0)	5.0d (4.1-6.6)
6	Jorhat	10	508.5cb (448.7-618.1)	14.6ab (14.0-15.0)	34.8cd (29.9-41.2)	11.2b (10.0-12.0)	3.5b (2.9-3.0)	84.3cde (67.0-117.0)	6.1ab (5.3-6.7)
7	Kalyani	12	545.0a (418.7-598.2)	13.9cd (13.0-15.0)	39.2a (29.6-44.5)	11.3b (10.0-12.0)	4.0a (3.0-5.0)	90.7bc (80.0-110.0)	6.2ab (4.8-6.7)
8	Kanpur	12	437.9d (398.8-468.6)	14.4abc (13.0-15.0)	30.4ef (26.6-33.0)	10.5c (10.0-11.0)	3.1bcd (3.0-4.0)	74.6fg (62.0-80.0)	5.9abc (5.4-6.4)
9	Ludhiana	11	441.4d (428.7-458.6)	14.8ab (14.0-15.0)	29.8ef (28.6-32.8)	11.3b (10.0-12.0)	3cd (3.0-3.0)	77.0def (69.0-86.0)	5.8abc (5.0-6.4)
10	Mandya	10	425.7d (349.0-468.6)	14.9a (14.0-16.0)	28.7f (21.8-31.3)	10.5c (10.0-11.0)	3.1bcd (2.0-4.0)	71.1g (67.0-75.0)	6.0abc (5.0-6.5)
11	New Delhi	16	542.1a (468.6-598.2)	14.4abc (13.0-16.0)	37.8ab (29.3-42.2)	11.3b (11.0-12.0)	3.4bc (3.0-4.0)	100.1a (89.0-118.0)	5.5cd (4.3-6.4)
12	Palampur	14	448.0d (428.7-458.6)	14.1bc (13.0-15.0)	31.8c (28.6-35.3)	10.4cd (10.0-11.0)	3.1bcd (2.5-4.0)	75.8ef (70.0-85.0)	6.0abc (5.3-6.4)
13	Samastipur	14	533.4ab (498.5-568.3)	14.9a (14.0-15.0)	35.8bc (33.2-39.9)	11.1b (10.0-12.0)	4.0a (3.0-5.0)	95.1ab (64.0-121.0)	5.8abc (4.6-8.6)
14	Vellayani	13	444.1d (428.7-458.6)	14.3abc (13.0-15.0)	31.1ef (28.6-34.5)	10.5c (10.0-11.0)	3.2bcd (3.0-4.0)	72.0g (68.0-81.0)	6.1ab (5.3-6.5)
	SE(m)		4.31	0.06	0.33	0.05	0.04	0.99	0.05
	CV %		12.17	5.37	13.14	6.73	17.28	15.64	10.83

PHX-G (µm)	β	EXC PORE (µm)	TL (µm)	c	ABW (µm)	ζ	HYL TL (µm)
161.8bcd (100.0-249.0)	3.1bcde (2.2-4.2)	81.9cb (70.0-95.0)	75.3cde (64.0-92.0)	6.4bc (6.1-6.8)	10.7abc (10.0-12.0)	7.1b (5.4-8.5)	21.6de (16.0-30.0)
137.4de (121.0-155.0)	3.2bcde (2.8-3.8)	73.8d (66.0-79.0)	67.5f (55.0-75.0)	6.4bc (6.0-7.2)	10.4bcd (10.0-11.0)	6.5bc (5.0-7.3)	19.25ef (16.0-22.0)
143.1cde (116.0-181.0)	3.8a (2.9-4.7)	87.9a (76.0-94.0)	76.0cd (62.0-83.0)	7.2a (6.1-8.2)	10.9ab (9.0-13.0)	7.0b (6.2-7.7)	22.5cde (18.0-26.0)
195.0a (105.0-242.0)	2.9cdef (2.2-4.2)	89.1a (66.0-98.0)	90.4a (70.0-102.0)	6.0d (5.4-6.5)	10.5abcd (10.0-12.0)	8.7a (5.8-10.2)	27.9a (18.0-32.0)
168.3abc (135.0-195.0)	2.7ef (2.3-3.4)	73.9d (71.0-78.0)	68.5f (58.0-73.0)	6.4bc (5.9-6.9)	11.1a (11.0-12.0)	6.2c (5.3-6.6)	19.1ef (15.0-21.0)
193.2a (121.0-272.0)	2.8def (2.0-3.7)	84.4b (72.0-105.0)	80.5bc (65.0-110.0)	6.4bcd (5.6-7.2)	10.1d (10.0-11.0)	8.0a (6.3-11.0)	24.3bc (18.0-35.0)
157.4bcd (124.0-230.0)	3.7ab (2.4-4.5)	86.4ab (65.0-96.0)	85.7ab (67.0-97.0)	6.4bcd (6.1-7.3)	10.6abcd (10.0-12.0)	8.2a (5.6-9.7)	26.5ab (19.0-31.0)
131.7de (110.0-160.0)	3.4abc (2.7-4.0)	73.8d (67.0-77.0)	69.6f (61.0-75.0)	6.4bcd (6.0-6.7)	10.3bcd (10.0-12.0)	6.7bc (6.1-7.5)	18.2f (17.0-20.0)
136.7de (109.0-179.0)	3.3abcd (2.6-4.1)	72.5d (66.0-78.0)	69.8def (62.0-77.0)	6.3bcd (5.7-7.1)	10.9ab (10.0-12.0)	6.4bc (5.6-7.3)	19.7ef (18.0-25.0)
175.2ab (130.0-198.0)	2.5f (2.0-3.1)	73.1d (64.0-78.0)	68.0f (42.0-79.0)	6.4bcd (5.6-8.3)	10.7abcd (10.0-12.0)	6.4bc (3.5-7.9)	19.9def (13.0-25.0)
187.9ab (128.0-244.0)	3.0cde (2.2-4.3)	89.2a (83.0-95.0)	85.9ab (80.0-97.0)	6.37bcd (5.6-6.9)	10.6abcd (10.0-12.0)	8.1a (7.3-9.4)	26.2ab (22.0-32.0)
124.8c (110.0-140.0)	3.6ab (3.1-4.1)	75.6d (68.0-80.0)	70.7def (68.0-75.0)	6.3bcd (6.0-6.6)	10.4bcd (10.0-12.0)	6.8bc (6.0-7.3)	19.4ef (17.0-22.0)
187.1ab (130.0-226.0)	2.9cdef (2.5-4.1)	88.5a (82.0-99.0)	87.4a (80.0-91.0)	6.1cd (5.8-7.0)	10.4bcd (10.0-11.0)	8.4a (7.8-9.0)	26.5ab (22.0-30.0)
124.8c (105.0-180.0)	3.7ab (2.5-4.2)	75.6d (72.0-79.0)	69.8def (65.0-75.0)	6.4bcd (6.0-6.7)	10.2cd (10.0-11.0)	6.8bc (6.5-7.3)	19.9def (17.0-23.0)
3.00	0.05	0.63	0.80	0.03	0.05	0.09	0.33
24.8	21.3	10.66	14.11	6.72	6.23	15.66	20.8

Mean values are followed by range and coefficient of variation (CV). Means within a column followed by the same letter are not different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 4. Measurements of males of different populations of *Meloidogyne grammicola*.

S. No.	Population	n	L (µm)	MBW (µm)	a	STYL. L. (µm)	DPGC (µm)	PHX-G (µm)
1	Anand	10	1,261.2ef (1,116.6-1,445.7) 7.81	30.4bc (28.0-33.0) 6.62	41.7cdef (36.8-51.6) 11.61	18.1bcd (17.0-19.0) 3.14	3.0fg (3.0-3.0) 0	-
2	Bhubaneswar	12	1,321.4de (1,195.6-1,505.5) 8.25	34.4a (30.0-40.0) 11.23	38.7ef (31.5-43.7) 10.32	17.24def (16.0-21.0) 8.74	3.0g (3.0-3.0) 0	218.8ab (187.0-248.0) 7.99
3	Hisar	11	1,738.4a (1,266.2-1,894.3) 9.69	31.1ab (30.0-35.0) 5.51	54.5a (42.2-61.1) 9.47	19.3a (18.0-20.0) 3.50	5.0a (4.0-6.0) 9.43	212.7abc (185.0-232.0) 8.03
4	Hyderabad	12	1,415.7cde (1,156.5-1,595.2) 12.45	31.2abc (27.0-37.0) 10.32	45.9bcd (38.6-56.1) 11.20	18.5abc (18.0-20.0) 3.64	4.8a (4.0-6.0) 11.95	232.5ab (231.0-234.0) 0.91
5	Jammu	11	1,313.3de (1,355.9-1,405.8) 7.09	32.9ab (33.0-34.0) 3.71	40.0def (39.9-42.6) 8.70	18.4abc (18.0-20.0) 2.83	3.7defg (3.0-4.0) 18.45	-
6	Jorhat	11	1,355.9de (1,116.6-1,595.2) 10.91	28.3c (22.0-33.0) 11.90	48.3b (36.3-54.5) 12.45	18.9ab (18.0-20.0) 4.02	3.9cdef (3.0-5.0) 18.92	213.0abc (213.0-213.0) 0
7	Kalyani	3	1,306.1de (1,296.1-1,316.0) 1.08	30.5bc (30.0-31.0) 2.32	42.9bcdef (41.8-43.9) 3.47	16.5f (16.5-16.5) 0	4.0bcde (4.0-4.0) 0	-
8	Kanpur	12	1,461.1bcd (1,296.1-1,675.0) 10.23	32.1ab (28.0-35.0) 8.97	45.6bcd (37.9-53.2) 8.18	18.1bcd (18.0-19.0) 1.67	3.4efg (3.0-5.0) 18.91	184.0c (162.0-203.0) 8.0
9	Ludhiana	10	1,118.6f (997.0-1,216.3) 6.85	29.6bc (26.0-32.0) 8.48	38.5f (34.9-46.0) 9.20	17.0ef (16.0-18.0) 6.32	3.7defg (3.0-4.0) 13.06	205.9bc (179.0-218.0)
10	New Delhi	10	1,538.4bc (1,146.6-1,894.3) 18.65	31.0bc (25.0-35.0) 13.10	48.9ab (38.5-59.2) 16.28	17.8cde (16.0-19.0) 3.98	4.4abcd (3.0-5.0) 18.81	240.0a (240.0-240.0)
11	Palampur	12	1,343.5de (1,176.5-1,475.6) 7.25	31.0bc (29.0-33.0) 5.00	43.3bcdef (38.7-49.9) 8.30	17.9bcde (17.0-18.0) 1.61	3.3fg (3.0-4.0) 13.92	221.ab (205.0-235.0) 4.90
12	Samasipur	6	1,615.2ab (1,435.7-1,844.5) 11.24	34.5a (30.0-41.0) 14.17	47.2bc (39.4-54.0) 10.96	19.4a (18.0-21.5) 6.18	4.7ab (4.0-5.0) 11.07	221.5ab (198.0-245.0) 15.00
13	Vellayani	10	1,383.8cde (1,176.5-1,724.8) 14.71	30.9bc (28.0-35.0) 6.36	45.0bcde (34.5-61.6) 20.53	18.6abc (17.0-20.0) 5.19	4.5abc (4.0-5.0) 11.88	-
	SE(m)		18.88	0.28	0.61	0.09	0.08	2.84
	CV%		15.25	10.07	15.11	5.68	21.96	8.97

b	EXC PORE (µm)	TL (µm)	c	ABW (µm)	ε	SPL (µm)	GUB (µm)
6.050c (5.7-6.6) 5.90	111.2def (101.0-130.0) 9.54	10c (9.0-11.0) 4.71	126.5bcd (108.8-150.7) 10.69	17.8b (17.0-19.0) 3.35	0.58ab (0.5-0.6) 7.27	28.2ef (26.5-30.0) 3.76	6.7abc (6.0-7.0) 7.21
8.1a (6.0-9.8) 14.74	116.2cdef (95.0-138.0) 10.18	10.7bc (10.0-13.0) 11.70	124.9bcd (95.1-150.5) 13.84	18.8ab (17.0-22.0) 8.97	0.58ab (0.5-0.7) 10.90	29.0fcd (27.0-31.0) 4.22	6.2c (6.0-7.0) 7.21
-	145.8a (120.0-188.0) 14.36	12.8a (10.0-20.0) 22.02	140.7bc (90.4-179.5) 20.85	20.0a (18.0-22.0) 6.71	0.65ab (0.5-0.9) 15.82	30.7ab (28.0-32.0) 3.52	7.0a (6.0-8.0) 6.70
-	129.2bc (105.0-145.0) 9.24	10.9bc (9.0-13.0) 10.98	130.2bcd (97.2-168.4) 16.64	15.9cde (14.0-18.0) 10.87	0.69a (0.6-0.8) 9.32	30.2abcd (28.0-32.0) 4.36	6.9abc (6.0-8.0) 11.35
-	111.1fde (104.0-108.0) 10.56	10.6bc (10.0-12.0) 9.65	124.2bcd (113.0-140.6) 9.67	17.5bcd (17.0-19.0) 9.71	0.61ab (0.6-0.6) 13.65	29.8abcde (28.0-31.0) 3.92	6.9abc (6.0-7.0) 10.14
5.6c (5.6-5.6) 0	107.9fe (92.0-132.0) 10.00	10.7bc (9.0-14.0) 13.88	128.8bcd (85.5-177.2) 21.78	17.5bcd (15.0-20.0) 9.37	0.63ab (0.5-0.8) 18.40	30.4abc (28.0-32.0) 3.91	6.3bc (6.0-7.0) 7.41
-	102.5f (100.0-105.0) 3.45	12.0ab (12.0-12.0) 0	108.9d (108.0-109.7) 1.10	19.0ab (18.0-20.0) 7.44	0.65ab (0.6-0.7) 10.88	28.5def (28.0-29.0) 2.48	6.5abc (6.0-7.0) 10.88
7.7ab (6.4-9.5) 17.23	109.0fde (90.0-121.0) 8.36	10.1bc (9.0-13.0) 13.57	147.5abc (128.8-175.0) 10.92	18.8ab (16.0-22.0) 10.28	0.54b (0.4-0.6) 12.95	28.6def (28.0-29.0) 1.81	6.6abc (6.0-7.0) 7.82
5.5c (4.8-6.7) 10.54	109.0fde (85.0-130.0) 14.03	9.3c (7.0-11.0) 17.77	123.2bcd (98.8-152.0) 17.40	15.1e (14.0-17.0) 6.98	0.61ab (0.5-0.7) 15.18	27.4f (26.0-29.0) 3.69	6.5abc (6.0-8.0) 11.63
5.6c (5.6-5.6) 0	124.9bcd (98.0-146.0) 15.20	10c (8.0-11.0) 13.09	154.0ab (119.6-181.5) 15.37	15.6de (15.0-17.0) 5.86	0.63ab (0.5-0.7) 14.18	31.5a (28.0-37.0) 9.61	6.9abc (6.0-8.0) 8.72
6.7bc (5.9-6.9) 6.41	123.0bcde (110.0-135.0) 5.85	11.2abc (9.0-13.0) 12.57	122.1cd (93.6-152.9) 14.36	19.0ab (16.0-22.0) 8.40	0.6ab (0.5-0.7) 14.21	28.1f (26.5-30.0) 3.80	6.6abc (6.0-7.0) 7.60
6.8abc (6.3-7.3) 10.48	135.2ab (115.0-168.0) 15.24	10.3bc (5.0-13.0) 27.82	170.0a (121.3-287.1) 36.24	17.3bcd (12.0-20.0) 16.98	0.6ab (0.4-0.8) 23.57	29.3bcde (28.0-32.0) 5.57	7.0ab (6.0-8.0) 9.04
-	112.1def (85.0-140.0) 13.98	10.2bc (7.0-13.0) 17.78	139.6bc (107.4-207.9) 24.12	17.6bc (14.0-20.0) 10.78	0.61ab (0.5-0.9) 21.09	28.4ef (26.0-32.0) 6.47	6.4abc (6.0-7.0) 8.18
0.19	1.53	0.16	2.43	0.19	0.01	0.16	0.06
19.06	14.54	16.58	20.05	12.40	15.48	6.15	9.16

Mean values are followed by range and coefficient of variation (CV). Means within a column followed by the same letter are not different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 5. Measurements of females of different populations of *Meloidogyne graminicola*.

S. No.	Population	n	L (µm)	MBW (µm)	a	Protuberance	STYL L (µm)	DPCO (µm)
1	Anand	12	476.9e (398.8-568.3)	299.1bcd (209.4-378.9)	1.6bc (1.1-2.1)	100%	11.8cd (11.0-13.0)	4.6cd (4.0-5.0)
2	Bhubaneswar	12	525.1cde (428.7-648.1)	304.1bcd (279.2-378.9)	1.7abc (1.5-2.2)	100%	10.8f (9.5-12.0)	5.1bc (3.5-7.0)
3	Hisar	12	525.1cde (428.7-648.1)	304.1bcd (279.2-378.9)	1.7abc (1.5-2.2)	100%	12.4b (12.0-13.0)	5.2b (5.0-6.0)
4	Hyderabad	12	567.5bcd (488.5-737.8)	332.3abc (239.3-488.5)	1.8abc (1.4-2.2)	100%	12.0bcd (11.5-12.5)	4.7bcd (4.0-5.0)
5	Jammu	11	501.2de (249.3-608.2)	264.7d (199.4-378.9)	2.a (0.7-2.7)	100%	11.9bcd (11.0-12.0)	4.6cd (4.0-5.0)
6	Jorhat	12	530.1bcde (428.7-707.9)	330.7abc (229.3-398.8)	1.6c (1.4-1.9)	100%	11.7cde (11.0-12.0)	4.4ed (4.0-5.0)
7	Kalyani	10	596.2bc (378.9-787.6)	358.9ab (219.3-568.3)	1.7abc (1.4-2.3)	100%	11.3ef (11.5-11.5)	4.3ed (4.0-5.0)
8	Kanpur	12	530.9bcde (418.7-658.0)	314.5bcd (259.2-398.8)	1.7abc (1.4-2.5)	100%	12.6bc (12.0-13.0)	4.6cd (4.0-5.0)
9	Ludhiana	11	532.1bcde (358.9-668.0)	287.3cd (199.4-368.9)	1.9abc (1.4-2.6)	100%	12.3bc (12.0-13.0)	4.4ed (4.0-5.0)
10	Mandya	12	541.7bcde (408.8-668.0)	317.0bcd (204.4-408.8)	1.8abc (1.3-2.2)	100%	11.5cd (11.0-12.0)	4.3ed (4.0-5.0)
11	New Delhi	11	697.0a (468.6-1,016.9)	386.1a (299.1-508.5)	1.8abc (1.5-2.2)	64%	13.6a (13.0-14.0)	5.9a (5.0-7.0)
12	Palampur	12	574.9bcd (418.7-687.9)	362.3ab (309.1-428.7)	1.6c (1.4-1.9)	100%	12.0bc (11.0-13.0)	4.6bcd (4.0-5.0)
13	Samastipur	12	612.7b (443.7-777.7)	340.7abc (244.3-548.4)	1.9abc (1.1-2.4)	100%	12.4b (12.0-15.0)	4.4ed (4.0-5.0)
14	Vellayani	11	569.3bcd (498.5-648.1)	307.1bcd (179.5-428.7)	2.0ab (1.5-2.8)	100%	9.3g (8.0-11.0)	15.81
	SE(m)		7.81	5.64	0.03		0.09	0.06
	CV %		17.87	22.23	18.12		8.95	15.75

EXC PORE (µm)	EPST ratio	VUL L (µm)	AVD (µm)	IPD (µm)	PP shape (Round/Oval)
22.2f (12.0-32.0)	1.88 (1.0-2.7)	25.9bcd (22.0-31.0)	17.8abc (15.0-22.0)	14.7abcde (11.0-20.0)	Round
26.8cde (18.0-32.0)	2.46 (1.6-3.0)	23.6d (20.0-27.0)	14.8d (13.0-16.0)	13.6de (11.0-16.0)	3/5
36.4b (32.0-41.0)	2.94 (2.7-3.4)	27.1bc (23.0-32.0)	19.2ab (16.0-24.0)	16.3ab (10.0-20.0)	9/2
33.7b (27.0-39.0)	2.57 (2.3-3.3)	24.8cd (20.0-33.0)	17.4abc (15.0-20.0)	12.8e (11.0-17.0)	6/4
25.4def (18.0-32.0)	2.13 (1.6-2.7)	26bcd (24.0-32.0)	17.9abc (14.0-20.0)	16.0abc (12.0-19.0)	4/6
29.1cd (25.0-36.0)	2.49 (2.1-3.0)	26.4bcd (20.0-34.0)	19.0ab (14.0-26.0)	14.0cde (10.0-18.0)	7/4
23.4ef (20.0-28.0)	2.08 (1.8-2.3)	28.1ab (25.0-33.0)	17.8abc (14.0-27.0)	16.7a (13.0-20.0)	9/1
24ef (15.0-28.0)	1.96 (1.3-2.3)	27bc (24.0-30.0)	17.6abc (13.0-20.0)	14.1bcde (8.0-16.0)	4/7
25.7cdef (24.0-28.0)	2.10 (1.8-2.3)	25.6bcd (23.0-27.0)	17.0bcd (15.0-20.0)	14.3bcde (12.0-20.0)	7/4
25.6cdef (21.0-35.0)	2.25 (1.8-3.2)	27.2bc (24.0-38.0)	17.5abc (15.0-20.0)	16.2ab (13.0-20.0)	10/3
40a (23.0-48.0)	2.93 (1.8-3.7)	30.8a (27.0-34.0)	19.5a (13.0-27.0)	14.9abcde (12.0-18.0)	Round
24.1ef (21.0-30.0)	2.00 (1.8-2.3)	24.5cd (19.0-30.0)	15.8cd (10.0-18.0)	13.2e (11.0-16.0)	Round
29.7c (24.0-41.0)	2.39 (1.9-3.2)	30.5a (25.0-35.0)	18.8ab (15.0-22.0)	15.455abcd (12.0-18.0)	9/2
28.4cd (25.0-32.0)	2.83 (2.5-4.0)	27.2bc (21.0-31.0)	16.2cd (13.0-19.0)	15.9abc (12.0-21.0)	Oval
		0.28	0.22	0.20	
		12.91	15.16	16.16	

Mean values are followed by range and coefficient of variation (CV). Means within a column followed by the same letter are not different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 6. Selection of variable in different stages of 14 Indian populations of *Meloidogyne graminicola*.

Variable IN/OUT	Status	Partial r^2	F	Pr > F	Wilks' Lambda	Pr < Lambda
Eggs L	IN	0.653	24.283	<0.0001	0.347	<0.0001
J2 L	IN	0.497	12.680	<0.0001	0.175	<0.0001
F STYL L	IN	0.429	9.590	<0.0001	0.100	<0.0001
J2 STYL L	IN	0.416	9.052	<0.0001	0.058	<0.0001
M DPGO	IN	0.401	8.463	<0.0001	0.035	<0.0001
M b'	IN	0.365	7.201	<0.0001	0.022	<0.0001
J2 DPGO	IN	0.321	5.890	<0.0001	0.015	<0.0001
F EXC PORE	IN	0.278	4.771	<0.0001	0.011	<0.0001
J2 b'	IN	0.266	4.469	<0.0001	0.008	<0.0001
J2 MBW	IN	0.266	4.439	<0.0001	0.006	<0.0001
Eggs W	IN	0.259	4.247	<0.0001	0.004	<0.0001
M ABW	IN	0.247	3.962	<0.0001	0.003	<0.0001
J2 c'	IN	0.227	3.526	<0.0001	0.003	<0.0001
F VUL L	IN	0.216	3.283	0.000	0.002	<0.0001
M PHX-G	IN	0.206	3.082	0.000	0.002	<0.0001
M SPL	IN	0.195	2.845	0.001	0.001	<0.0001
J2 b'	IN	0.194	2.815	0.001	0.001	<0.0001
M c	IN	0.186	2.654	0.002	0.001	<0.0001
M STYL L	IN	0.171	2.373	0.006	0.001	<0.0001
M MBW	IN	0.174	2.411	0.006	0.001	<0.0001
M EXC PORE	IN	0.164	2.237	0.011	0.000	<0.0001
F DPGO	IN	0.169	2.307	0.008	0.000	<0.0001
F IPD	IN	0.159	2.128	0.016	0.000	<0.0001
F L	IN	0.140	1.821	0.045	0.000	<0.0001
J2 PHX-C	IN	0.141	1.818	0.045	0.000	<0.0001

Euclidean distance coefficients, the agglomerative hierarchical clustering (AHC) dissimilarity using Ward's method and phenological dendrograms was constructed to evaluate the level of phenotypic variation in different stages among the 14 populations of *M. graminicola*. The data were run through AHC to investigate the separability of the 14 populations based on their mean morphometric characters. The hierarchical cluster based on 42 morphometrical characters of eggs, J2, males, and females were examined together, and the distance index (Fig. 2) showed that the 14 populations of *M. graminicola* can be grouped into four main clusters on the basis of animated truncation (dotted line). Cluster 1 (C1) comprised of Anand, Bhubaneswar,

Jammu, Ludhiana, and Mandya populations; Cluster 2 (C2) included only Hisar population; Cluster 3 (C3) constituted Hyderabad, Jorhat, Kalyani, Kanpur, Palampur, and Vellayani populations; whereas Cluster 4 (C4) comprised of New Delhi and Samastipur populations. The within class variance values among the clusters 1, 3, and 4 were not highly variable (Table 8).

The discriminant analysis performed with standardized canonical discriminant function coefficients for 42 morphometrical characters showed that F1 alone accounted for 29.895% variation, and along with F2, it showed 43.893% variability. First eight functions contributed to 90.941% variation among the populations (Table 9).

TABLE 7. Proximity matrix (Euclidean distances) between 14 Indian populations of *Meloidogyne graminicola*.

POPUL ^N	AND	BHU	HSR	HYD	JAM	JOR	KAL	KAN	LDH	MAD	DEL	PAL	SAM	VEL
AND	0.00	98.01	484.49	197.51	83.34	121.15	155.67	218.84	162.45	115.14	373.50	152.91	389.56	164.68
BHU	98.01	0.00	431.31	169.31	58.83	107.98	151.37	146.96	204.62	135.08	318.21	83.04	334.53	81.41
HSR	484.49	431.31	0.00	332.93	441.27	389.71	446.18	298.60	628.74	555.54	283.23	413.44	166.12	371.46
HYD	197.51	169.31	332.93	0.00	180.55	85.36	130.54	151.93	327.77	255.71	188.52	146.17	209.43	136.04
JAM	83.34	58.83	441.27	180.55	0.00	115.11	175.01	168.51	201.85	138.87	343.49	135.11	350.13	118.23
JOR	121.15	107.98	389.71	85.36	115.11	0.00	105.85	147.95	257.80	185.27	259.90	110.80	278.56	112.47
KAL	155.67	151.37	446.18	130.54	175.01	105.85	0.00	214.35	238.64	185.37	264.03	117.36	320.24	150.62
KAN	218.84	146.96	298.60	151.93	168.51	147.95	214.35	0.00	345.36	271.08	240.87	143.05	217.59	93.95
LDH	162.45	204.62	628.74	327.77	201.85	257.80	238.64	345.36	0.00	98.92	479.50	242.31	520.82	270.24
MAD	115.14	135.08	555.54	255.71	138.87	185.27	185.37	271.08	98.92	0.00	402.90	169.60	441.75	197.94
NDL	373.50	318.21	283.23	188.52	343.49	259.90	264.03	240.87	479.50	402.90	0.00	263.26	126.57	250.99
PAL	152.91	83.04	413.44	146.17	135.11	110.80	117.36	143.05	242.31	169.60	263.26	0.00	300.88	73.17
SAM	389.56	334.53	166.12	209.43	350.13	278.56	320.24	217.59	520.82	441.75	126.57	300.88	0.00	267.51
VEL	164.68	81.41	371.46	136.04	118.23	112.47	150.62	93.95	270.24	197.94	250.99	73.17	267.51	0.00

TABLE 8. Variance among four classes of 14 Indian populations of *Meloidogyne graminicola*.

Class	C1	C2	C3	C4
Objects	5	1	6	2
Sum of weights	5	1	6	2
Within-class variance	9,479.492	0.000	8,744.988	8,009.809
Minimum distance to centroid	60.463	0.000	59.975	63.284
Average distance to centroid	83.917	0.000	82.910	63.284
Maximum distance to centroid	127.178	0.000	112.901	63.284
	Anand	Hisar	Hyderabad	New Delhi
	Bhubneshwar		Jorhat	Samastipur
	Jammu		Kalyani	
	Ludhiana		Kanpur	
	Mandya		Palampur	
			Vellayani	

Of 14 “*M. graminicola*” populations studied, it was clearly revealed both by conventional statistical analysis (DMRT) as well as advanced statistical method (AHC), that three populations namely Hisar, New Delhi, and Samastipur appear quite different from typical *M. graminicola*.

It was, therefore, thought pertinent to elucidate their affinity with closely related species, particularly, *M. graminis* Maas, Sanders and Dede, 1978 (reported from weed); *M. oryzae* Maas, Sanders and Dede, 1978; *M. salasi* Lopez, 1984; *M. triticoryzae* Gaur, Saha and Khan, 1993; and *M. lini* Yang, Hu and Xu, 1988. The morphometrical details in respect of *M. hainanensis* were

not available unfortunately. The 12 available morphometrical parameters of J2, males and females pertaining to these species were taken from their original descriptions (Table 10) and comparable characteristics of 14 populations under investigation were subjected to AHC dissimilarity through Euclidean distance coefficients using Ward’s method. The results classified the populations of “*M. graminicola*” and five closely related species into three broad groups: (i) Anand, Bhubaneswar, Hyderabad, Jammu, Jorhat, Kalyani, Kanpur, Ludhiana, Mandya, Palampur, and Vellayani along with *M. graminicola*, *M. triticoryzae*, *M. salasi*, (ii) Hisar, New Delhi, Samastipur along with *M. oryzae*, *M. graminis*, and

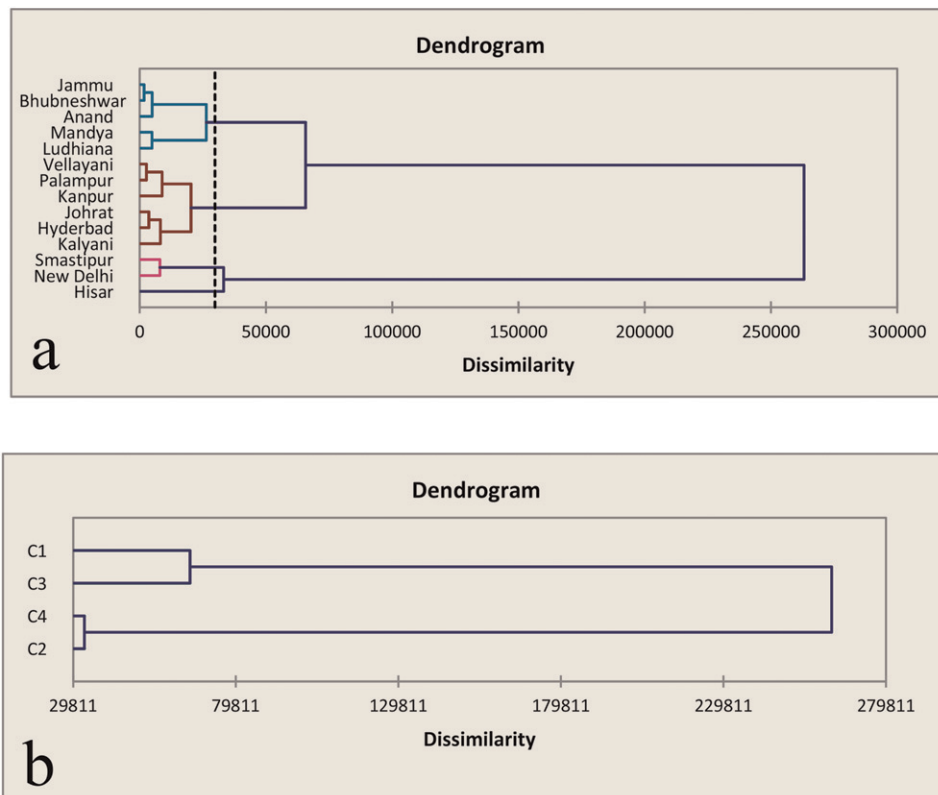


FIG. 2. Dendrograms of 14 Indian populations of *Meloidogyne graminicola* on the basis of phenotypic variation in different stages.

TABLE 9. Eigenvalues.

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Eigenvalue	4.321	2.023	1.970	1.602	1.088	0.880	0.697	0.564	0.387	0.341	0.257	0.190	0.135
Discrimination (%)	29.895	13.998	13.631	11.085	7.524	6.087	4.820	3.901	2.674	2.359	1.778	1.315	0.932
Cumulative percentage	29.895	43.893	57.524	68.609	76.133	82.220	87.040	90.941	93.616	95.975	97.753	99.068	100.000

(iii) *M. lini* (Fig. 3). Furthermore, it is also revealed that this discrimination into three clusters is mainly resolved by total body lengths of J2, males and females.

Molecular phylogenetic analysis: The ITS sequences of Indian *M. graminicola* isolates obtained and used in this study were deposited in GenBank (accession nos. MF320120 to MF320127); the molecular phylogenetic analysis is represented in Fig. 4. We were able to amplify ITS from eight of 14 populations. The phylogenetic analysis and comparison with other *Meloidogyne* sequences obtained from GenBank revealed that all the Indian populations are grouped into *M. graminicola* clade. The ITS marker failed to resolve the presence of subgroups within the Indian populations of *M. graminicola*.

DISCUSSION

In the present study, 14 populations of *M. graminicola* were collected from various parts of the country representing diverse agroecological zones. Although the populations were procured and supplied under the presumption of them being *M. graminicola*, the possibility of occurrence of closely related species was not ruled out while conducting the present study. The selection of 42 morphometrical and morphological

characteristics for all stages was based on the important parameters previously used in this taxonomic group (*Meloidogyne*).

Comparisons of CV values within the individuals of a population and among the populations were used to identify the least variable and highly variable characteristics. CV values of egg length and width were quite low—indicating stability of the parameters within populations. Certain J2 morphometric values, e.g., the distance from the anterior end up to gland overlap, *b'*-value, length of hyaline portion of tail and *c'*-value were highly variable in some populations. Least variation was observed for characters like stylet length, distance of excretory pore from the anterior end, maximum body width, *a*-value, *b*-value, and anal body width. Among males, stylet length, spicule length, and gubernaculum length were least variable, whereas characters like tail length, *c*-value, *c'*-value were highly variable in Hisar, Jorhat, Samastipur, and Vellayani populations. Among female characteristics, stylet length and vulval length were most stable within populations.

Based on the DMRT application, it clearly emerged that Hisar, New Delhi, and Samastipur populations of “*M. graminicola*” were distinctly different from the remaining 11 populations. The major characteristics that

TABLE 10. Morphometrical characteristics of different populations of “*Meloidogyne graminicola*” along with other closely related nominal species.

Sr. No.	Populations or species	J2								F			M				
		STYL L	L	<i>a</i>	<i>b'</i>	TL	HYL TL	<i>c</i>	<i>c'</i>	L	STYL L	DPGO	L	<i>a</i>	STYL L	SPL	GUB
1	Anand	11.43	484.55	32.43	3.14	75.27	21.60	6.45	7.07	476.90	11.80	4.60	1,261.21	41.70	18.10	28.20	6.70
2	Bhubaneswar	11.83	432.05	32.14	3.17	67.46	19.25	6.41	6.52	525.08	10.83	5.13	1,321.39	38.70	17.23	29.00	6.20
3	Hisar	11.81	531.86	37.22	3.78	76.00	22.50	7.17	7.00	525.08	12.36	5.18	1,738.41	54.53	19.30	30.73	7.05
4	Hyderabad	11.59	540.20	36.96	2.86	90.36	27.91	6.00	8.63	567.46	11.96	4.68	1,415.74	45.88	18.50	30.20	6.89
5	Jammu	10.70	437.69	29.81	2.65	68.50	19.10	6.39	6.19	501.24	11.91	4.60	1,313.33	39.99	18.46	29.82	6.91
6	Jorhat	11.20	508.49	34.80	2.79	80.50	24.30	6.37	7.99	530.08	11.73	4.36	1,355.92	48.34	18.95	30.38	6.25
7	Kalyani	11.33	545.02	39.23	3.65	85.67	26.50	6.37	8.15	596.21	11.25	4.25	1,306.05	42.85	16.50	28.50	6.50
8	Kanpur	10.50	437.86	30.42	3.38	69.00	18.17	6.36	6.69	530.90	12.25	4.58	1,461.06	45.62	18.09	28.60	6.60
9	Ludhiana	11.27	441.41	29.80	3.32	69.80	19.70	6.34	6.42	532.05	12.27	4.36	1,118.64	38.52	17.00	27.44	6.50
10	Mandya	10.45	425.73	28.65	2.45	68.00	19.90	6.35	6.42	541.70	11.46	4.27	1,196.40	37.39	17.00	29.00	6.50
11	New Delhi	11.25	542.13	37.79	3.02	85.93	26.19	6.37	8.12	697.01	13.60	5.90	1,538.38	48.93	17.80	31.50	6.89
12	Palampur	10.39	447.95	31.76	3.60	70.69	19.36	6.33	6.83	574.93	12.05	4.64	1,343.48	43.32	17.92	28.09	6.64
13	Samastipur	11.14	533.39	35.76	2.94	87.36	26.50	6.12	8.39	612.74	12.42	4.42	1,615.17	47.22	19.42	29.33	7.00
14	Vellayani	10.46	444.08	31.07	3.66	69.85	19.92	6.35	6.83	569.29	9.30	4.00	1,383.84	44.96	18.60	28.40	6.44
15	<i>M. graminicola</i>	11.04	481.78	33.40	3.14	75.73	22.09	6.38	7.21	554.70	11.81	4.64	1,395.21	44.77	18.20	29.28	6.70
16	<i>Meloidogyne triticoryzae</i>	12.10	395.70	36.40	3.65	61.70	17.60	6.44	6.17	425.00	13.20	2.90	1,305.00	63.50	17.50	29.00	9.75
17	<i>Meloidogyne oryzae</i>	14.2	545	37	8.2	79	21	7	7.8	625	15	7	1,667	56	19	29.5	9
18	<i>Meloidogyne graminis</i>	12.61	475	31.74	8.1	76	18.9	6.07	7.7	726.00	12.46	4.1	1,512	43.5	18.31	28.26	8.12
19	<i>Meloidogyne salasi</i>	11	418	27	-	-	-	7	6.3	588	13	3.1	1,069	44	15	22	7
20	<i>Meloidogyne lini</i>	16.1	478	29.8	8.1	60.5	-	7.9	-	933	12	3	2,242	35	18	37.9	9.5

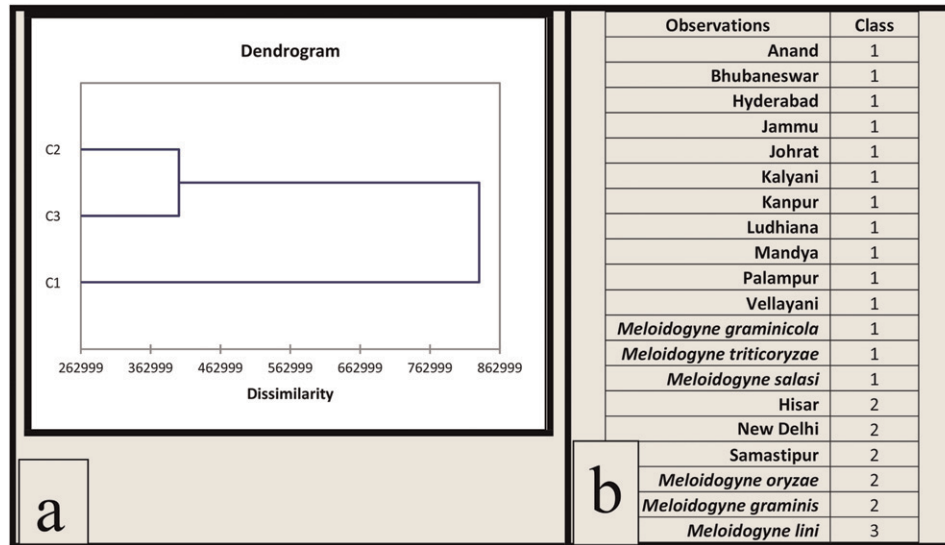


FIG. 3. Dendrogram of 14 populations of “*Meloidogyne graminicola*” characteristics along with closely related *Meloidogyne* spp. A. Final grouping; B. Grouping table.

discriminated these populations included length of eggs; J2 length, a -value, hyaline tail portion; male length, distance up to excretory pore, spicule and gubernaculum lengths; female length and width, stylet length, distance up to excretory pore, EPST ratio and vulval length. A comparison of the morphometrical characteristics in the descriptions of *M. graminicola* by Golden and Birchfield (1965) and subsequently by Mulk (1976) revealed the distinctiveness of Hisar, Samastipur, and New Delhi populations. Shape and size of the females, inconsistency in the presence of vulval protuberance, and lack of lateral alae in the males were other unique characters observed particularly in the New Delhi population. Intraspecific variations recorded on morphological basis as per Pokharel et al. (2007) show that J2 of Bangladesh and the United States were significantly longer and smaller, respectively, than the Nepal isolates collected from 33 different locations; besides, minor variability was also observed among the Nepalese isolates. A significant correlation ($P = 0.0025$) was observed only between the stylet length and body length. In addition, a , b , and c values did not correlate with each other or with the body length. The perineal patterns of the Nepalese isolates were dorsoventral, oval to almost circular in shape, moderate in height of arc, and no lateral incisures or gaps were observed. The tail tip was marked with prominent, coarse, fairly well-separated striae that sometimes formed an irregular tail whorl. These perineal patterns were similar to the pattern described for *M. graminicola*, with some minor variations and overlap with those of *M. oryzae* and *M. trifoliophila* Bernard and Eisenback, 1997. In another study, Pokharel et al. (2010) compared 10 isolates of *M. graminicola* from broad geographic

areas; variation observed in morphometric measurements among and within isolates did not correlate with the geographic source of the isolates.

In the present study, the application of DMRT and multivariate analysis in resolving *M. graminicola* populations thus clearly established the distinctiveness of three populations (New Delhi, Hisar, and Samastipur) compared with the rest, the relative importance of characteristics applied by the two programs notwithstanding. Whereas DMRT identifies the parameters used for segregating populations, their relative weightage in imparting such segregation is possible by the discriminant analysis performed with standardized canonical discriminant function coefficients. Thus, it can be deduced that of 13 shortlisted variables, egg length alone accounted for 29.895% variation, whereas J2 total body length contributed 13.998% variation among the populations.

This analysis of *M. graminicola* “group of species” used in this study with closely related species has led to some interesting revelations: (i) *M. graminicola*, *M. triticoryzae*, and *M. salasi* are allied (synonymous?) species, and a majority (11) of the populations in the present study belong to this category; (ii) *M. oryzae* and *M. graminis* are closely related (synonymous?) species, but completely divergent from *M. graminicola*; (iii) the observed dissimilarity of Hisar, New Delhi, and Samastipur populations from the remaining 11 populations used in this study is vindicated; (iv) Hisar, New Delhi, and Samastipur populations belong to *M. oryzae* or *M. graminis* subgroup, instead of *M. graminicola*; and (v) *M. lini* is entirely different, and constitutes a third category. Validity of *M. triticoryzae* has been questioned by many taxonomists (Khan et al., 2014).

To investigate the molecular basis of morphological variations, we reconstructed the phylogeny of the

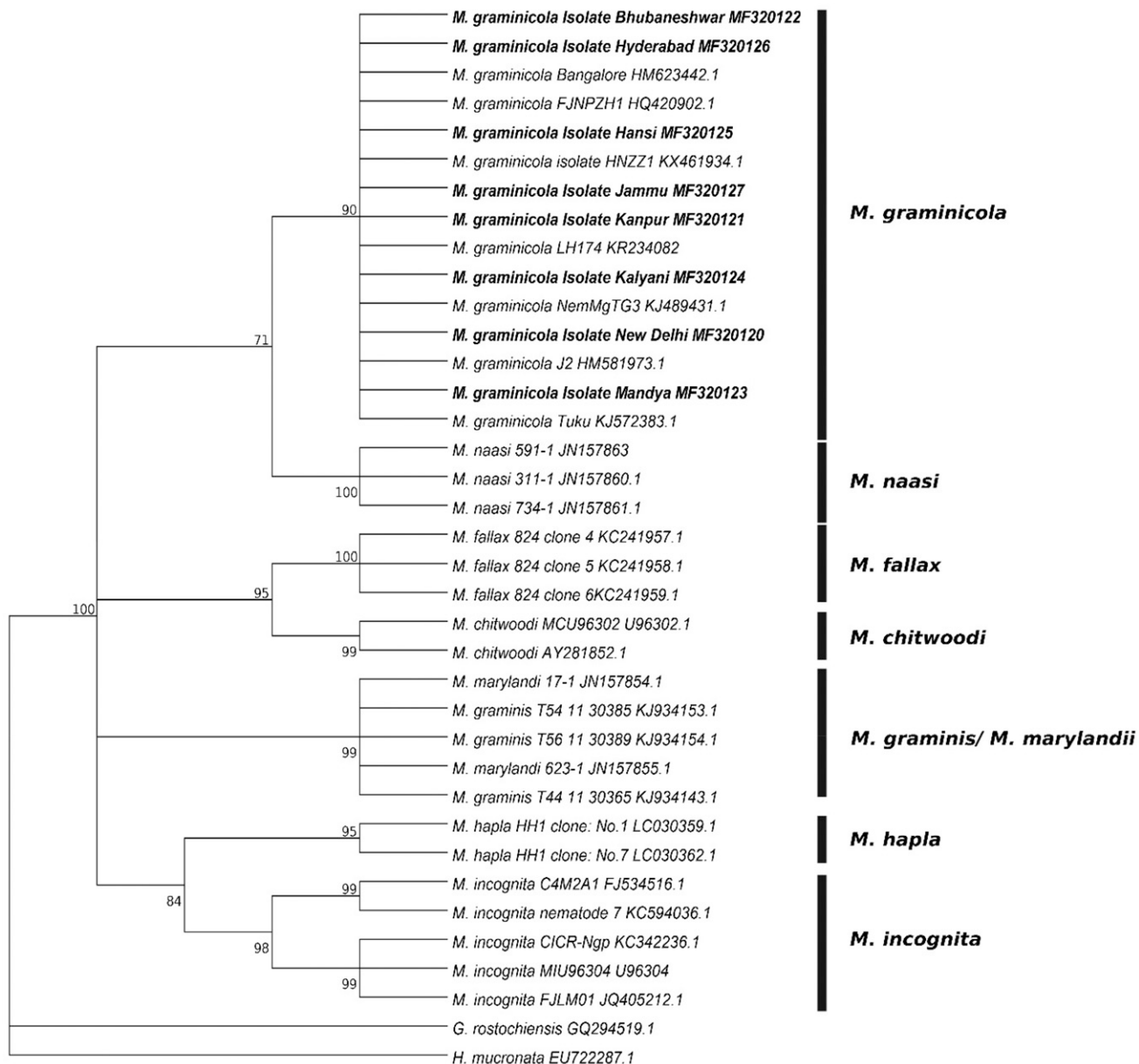


FIG. 4. A molecular phylogenetic analysis of Indian *Meloidogyne graminicola* populations based on the ITS sequences by maximum likelihood method using general time reversible model. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed. The bootstrap values (1,000 replicates) are shown next to the branches. Branches corresponding to partitions reproduced in less than 60% bootstrap replicates were collapsed to get a robust tree. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with superior log likelihood value. A discrete gamma distribution was used to model evolutionary rate differences among sites (five categories [+G, parameter = 2.4321]). The analysis involved 37 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. There were a total of 849 positions in the final dataset. The Indian populations isolated and used in this study are highlighted in bold. The GenBank accession numbers of the sequences obtained from GenBank and used for phylogenetic analysis are indicated next to *Meloidogyne* spp. name. Evolutionary analyses were conducted in MEGA6.

M. graminicola populations using ITS marker. In spite of morphological and morphometrical differences, the ITS marker-based molecular analysis revealed that all the nematodes belonged to one species—*M. graminicola*. It is possible that these morphometrical differences may be because of geographical intraspecific variability not associated with a molecular marker, presenting another example of either phenotypic plasticity exhibited by nematodes (Viney and Diaz,

2012; Nijhout, 2015; de Oliveira et al., 2017), or the presence of races within *M. graminicola* (Pokharel et al., 2010; Bellafiore et al., 2015). In a previous study, 10 different geographical isolates of *M. graminicola* showed substantial variation in morphometric measurements, yet the ITS sequences of all 10 isolates identified them as *M. graminicola* (Pokharel et al., 2010). Several studies (Jepson, 1983; Pokharel et al., 2007, 2010; Bellafiore et al., 2015) used morphometrical characters to reveal

intraspecific population diversity between *M. graminicola* populations in Vietnam, Nepal, India, Thailand, and the United States. Coupled with host pathogenicity studies, it was suggested that *M. graminicola* populations could consist of more than one race.

Identification and diagnosis of *M. graminicola* has always been a challenge (Hunt and Handoo, 2009), and many markers based on nuclear or mitochondrial DNA have been used including the ITS region (Pokharel et al., 2007; Besnard et al., 2014; Bellaifiore et al., 2015). It has been suggested that mitochondrial phylogenetic markers including variable number tandem repeats could help resolve the population diversity better than nuclear markers (Lunt et al., 1998; Besnard et al., 2014; Sun et al., 2014; Humphreys-Pereira and Elling, 2015). Testing additional phylogenetic markers would resolve if our populations belong to *M. graminicola* or to other cryptic species.

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