

Case Study

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Morphological and Morphometric Variations among the Population of Root-Knot Nematode(s) of Assam

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ABSTRACT

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Study on the morphological variations among the populations of *Meloidogyne incognita* from nine districts of Assam exhibited variations only in the tail shape, while other morphological characters showed similarities among the populations. Tail shape of second stage juveniles (J_2) of *M. incognita* collected from Golaghat, Kokrajhar, Nagaon and Sibsagar district was similar in shape. Likewise, J_2 tail shape of Dibrugarh, Jorhat and Lakhimpur population showed similarity but, Dhubri and Kamrup population showed variation from other populations. No variation was observed in size and shape of perineal pattern of *M. incognita* collected from nine districts of Assam. Studies on morphometric variations (body length, stylet length, lip height, lip width, MB, a, b, b', c and c') among the populations of *M. incognita* from nine districts of Assam showed no distinct variations in the major characters, except in MB value. MB value of Kamrup population was higher (61.79) than other populations.

Introduction

Nematodes are highly diversified, ubiquitous group of invertebrates that occurs in all possible kind of climatic conditions and habitats. Until middle of the last century, plant parasitic nematodes did not get worldwide recognition as important causal agents of plant diseases, but are now realized as a great menace in successful cultivation of crops, limiting production, productivity and quality as well. Plant parasitic nematodes are responsible for 12.3 per cent average annual loss of the world's major crops. Their threat being more severe in underdeveloped and

developing countries with warm and humid climate than developed countries that lie in the temperate zones. It is evident from the fact that an average loss of 14.6 per cent was estimated due to plant parasitic nematodes in developing countries as compared to 8.8 per cent in developed countries (Sasser and Freckman, 1987). In India, the loss on crops due to plant parasitic nematodes goes up to 10 per cent (Dasgupta, 1998). Among the important economic plant parasitic nematodes, *Meloidogyne* spp. ranks first, causing a great loss to the crops ranging upto 90.9 per cent in okra (Bhatti and Jain, 1977). Due to many biotic and abiotic influences, morphological

and morphometric characters have been found to be altered among this nematode. Geographical variation is also considered as one of the major cause for morphological and morphometric variations. Considering the importance of accurate identification of root-knot nematode for proper management strategies the following work has been carried out.

Soil along with root samples were collected from around the rhizosphere of vegetable crops from nine districts of Assam viz., Golaghat, Kokrajhar, Nagaon, Sibsagar, Dibrugarh, Jorhat, Lakhimpur, Dhubri and Kamrup. Root-knot nematode population collected from these nine districts were extracted from soil as well as from root using Cobb's sieving and decanting method and Baermann funnel technique, respectively. Nematode suspension, concentrated in few ml of water were killed and fixed simultaneously by pouring equal amount of warm 8 per cent formalin (8ml formaldehyde solution + 92 ml distilled water). Clearing of nematodes were done using Seinhorst's method (Seinhorst, 1962). Paraffin waxes were used for mounting and sealing of nematodes for permanent slide preparation. For preparation of perineal pattern, entire knotted root system was cut into a small pieces and stained by NaOCl-Acid Fuchsin method (Byrd *et al.*, 1983). Ten mature females from each district were taken for preparation of perineal pattern. Measurements of the mounted nematode were taken with the help of an ocular micrometer, calibrated with a stage micrometer. For denoting the dimensions of nematodes, de Man's (1880) formulae were used.

Morphology

Mature female (n=10): Body pearly white, globular to pear shaped, with projecting neck. Cuticle translucent and glistening. Annulations visible in neck and vulva-anus region. Lip set-off. Stylet slender with distinct rounded knobs. Esophagus well developed with large

cylindrical procorpus and rounded metacarpus. Excretory pore just behind the level of stylet knobs. Perineal pattern wavy dorsally. Dorsal arch high and squarish. Perineal pattern of *Meloidogyne* spp. collected from nine districts of Assam were found to be similar with the perineal pattern of *M. incognita* with high dorsal arch, distinct lateral lines absent. Vulva, anus and phasmid present in perineum. No variation on perineal pattern was observed in size and shape of perineal pattern of *M. incognita* collected from nine districts of Assam (Fig. 1).

J₂(n=15): Body slender, lip region continuous with the body contour. Cephalic framework well developed. Stylet slender with distinct round small basal knobs. Metacarpus distinct with basal bulb extension overlapped ventrally, genital primordia developed, rectum and anus clearly visible. Tail with hyaline terminus conoid shaped. Morphological characters of J₂ *M. incognita* population collected from nine districts of Assam exhibited similarity in head portion (Fig. 2) with variations in tail shape (Fig. 3). Similar tail shape of J₂*M. incognita* collected from Golaghat, Kokrajhar, Nagaon and Sibsagar district was recorded. Likewise, tail shape of J₂ of Dibrugarh, Jorhat and Lakhimpur; Dhubri with Kamrup population showed similarity.

Morphometric

J₂(n=15): Populations of *M. incognita* from nine districts of Assam showed no distinct variations in the major characters, except in MB value. MB value of Kamrup population was higher (61.79) than other populations.

Table 1 and 2 showed that from all the nine populations of *M. incognita* J₂ collected, longest body length were found in Kamrup (0.478 mm) while, shortest being in Golaghat (0.360 mm). Lip height, lip width and stylet length were in same range with 2-3 μ m, 4-5

µm and 10-12 µm, respectively. Length from the anterior end to the centre of median bulb in relation to esophagus length (MB) showed variation. Highest value of MB was recorded in Kamrup(61.79) followed by Jorhat (54.63) while, lowest being recorded in Sibsagar (36.36). Highest a and b ratio was recorded in Sibsagar (33.38) and Golaghat (5.61) while,

lowest was recorded in Golaghat(26.07) and Sibsagar (3.81), respectively. Highest b' ratio was recorded in Lakhimpur (4.48) while, lowest being found in Jorhat (3.01). Highest c and c' ratio was recorded in Kokrajhar (10.66) and Lakhimpur (4.90), while lowest being found in Lakhimpur (7.73) and Kokrajhar (3.00), respectively.

Table.1 Comparison of morphometric data of *Meloidogyne incognita* (J₂) from nine districts of Assam

Characters	Jorhat	Golaghat	Sibsagar	Lakhimpur	Dhubri	Nagaon	Dibrugarh	Kokrajhar	Kamrup
J ₂	n=15								
L	0.366-0.434mm (0.398±0.225)	0.360-0.440mm (0.410±0.234)	0.382-0.473mm (0.390±0.030)	0.372-0.459mm (0.426±0.291)	0.373-0.451mm (0.411±0.023)	0.364-0.449mm (0.405±0.024)	0.382-0.422mm (0.400±0.013)	0.384-0.448mm (0.410±0.023)	0.417-0.478mm (0.440±0.020)
Stylet Length	10.0-12.0µm (11.5±0.6)	10.0-12.0µm (10.8±0.7)	10.0-12.0µm (11.0±0.6)	10.0-12.0µm (11.0±0.5)	10.0-12.0µm (11.2±0.7)	10.0-12.0µm (10.8±0.05)	10.0-12.0µm (11.5±0.06)	10.0-12.0µm (11.2±0.79)	10.0 - 12.0µm (11.2±0.72)
Lip height	2.0µm	2.0µm	2.0µm	2.0-3.0µm (2.0±0.02)	2.0µm	2.0µm	2.0µm	2.0µm	2.0 µm
Lip width	4.0µm	4.0-5.0µm (4.2±0.04)	4.0-5.0µm (4.3±0.04)	4.0-5.0µm (4.6±0.04)	4.0 -5.0µm (4.5±0.51)	4.0-5.0µm (4.6± 0.05)	4.0µm	4.0 µm	4.0 -5.0µm (4.6±0.04)
a	26.14-33.30 (29.96±1.17)	26.07-32.53 (30.71±1.61)	27.78-33.38 (30.80±2.74)	28.61-30.64 (29.87±0.67)	27.71-30.53 (29.13±0.89)	28.00-30.71 (29.45±0.69)	27.64-30.23 (29.02±0.93)	27.92-31.07 (29.74±0.85)	29.33-31.86 (30.33±0.81)
b	4.11-5.41 (4.87±0.36)	3.95-5.61 (4.69±0.42)	3.81-4.91 (4.80±0.67)	4.59-5.20 (5.01±0.19)	4.82 -4.96 (4.88±0.04)	4.78-4.97 (4.89±0.04)	4.22-4.66 (4.41±0.15)	4.82-5.30 (5.02±0.14)	4.96-5.27 (5.05±0.13)
b'	3.01-3.89 (3.53±0.41)	3.08-3.79 (3.44±0.18)	3.05-4.01 (3.49±0.67)	3.17-4.48 (3.59±0.37)	3.46 -4.12 (3.71±0.33)	3.15-4.10 (3.52±0.32)	3.39-3.63 (3.53±0.08)	3.47-3.50 (3.48±0.02)	3.46-4.35 (3.87±0.31)
c	8.77-9.64 (9.16±0.30)	8.29-9.84 (9.28±0.47)	8.64-10.50 (9.23±0.59)	7.73-9.2 (8.47±0.48)	8.22-9.43 (8.82±0.27)	8.66-9.55 (9.09±0.31)	8.88-9.28 (8.98±0.13)	9.00-10.66 (9.90±0.55)	9.00-10.15 (9.43 ±0.36)
c'	3.75-4.40 (3.95±0.17)	3.75-4.50 (4.10±0.22)	3.50-4.18 (3.96±0.27)	4.00-4.90 (4.39±0.28)	3.66 - 4.16 (3.83±0.18)	3.58-4.09 (3.89±0.18)	3.58-4.00 (3.77±0.11)	3.00-4.08 (3.57±0.35)	3.46-4.00 (3.76±0.14)
MB	41.04-54.63 (46.42±5.84)	42.18-53.09 (46.99±4.79)	36.36-49.07 (46.61±4.59)	46.15-53.71 (50.42±2.62)	44.00-54.16 (48.98±4.46)	42.27-53.92 (48.90±4.17)	38.63-49.53 (45.77±4.56)	40.75-45.16 (43.83±1.85)	39.06-61.79 (51.87±6.39)

*data in parenthesis is mean ± SD

Table.2 Comparison of morphometric data of *Meloidogyne incognita* (J₂) with Kofoid and White (1919), Whitehead (1968), Khan and Murmu (2006) and Das (1993)

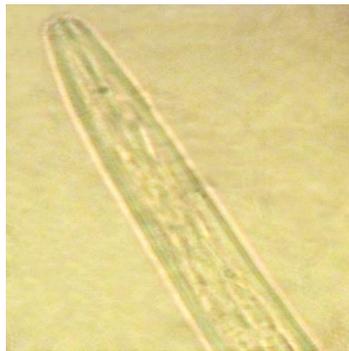
Characters	Kofoid and White (1919)	Whitehead (1968)	Khan and Murmu (2006) works on West Bengal	Das (1993) works on Assam	Present work on Assam populations
Second stage juvenile				n=10	n=15
L	0.36- 0.393 mm	0.337-0.403 mm	0.327-0.471 mm (0.404)	0.40-0.43 mm	0.360-0.478 mm (0.414±0.01)
Stylet Length	10 µm	9.6-11.7 µm (10.5)	11.88-13.86 (13.32)	10.0-12.0 µm	10.0-12.0 µm (11.0±0.6)
Lip height	-	-	-	-	2.0 µm
Lip width	-	-	-	-	4.0-5.0 µm (4.31±0.26)
A	29.0-33.0	24.9-31.5 (28.5)	23.60-34.76 (28.42)	24.0-29.0 (25.5)	26.07-33.38 (29.89±0.63)
B	5.6-6.4	2.02-3.14 (2.36)	3.94-5.08 (4.49)	2.5-3.0 (2.7)	3.81-5.61 (4.84±0.19)
b'	-	-	-	-	3.01-3.48 (3.57±0.135)
c	8.0-9.4	6.9-10.6 (8.1)	6.16-8.70	8.12-9.2 (8.52)	7.73-10.66 (9.15±0.39)
c'	-	-	-	-	3.00-4.90 (3.91±0.23)
MB	-	-	-	-	36.36-61.79 (47.54)

*data in parenthesis is mean ±SD

Fig.1 Perineal pattern of *M. incognita*



Fig.2 Head portion of *M. incognita* J₂ from nine districts of Assam (100X)



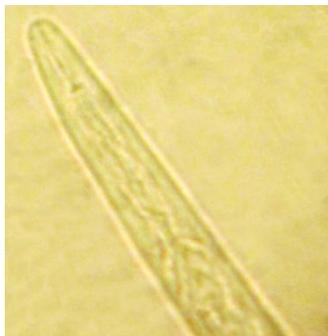
Dhubri



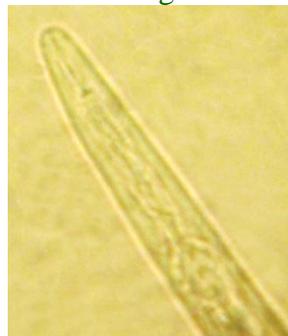
Dibrugarh



Golaghat



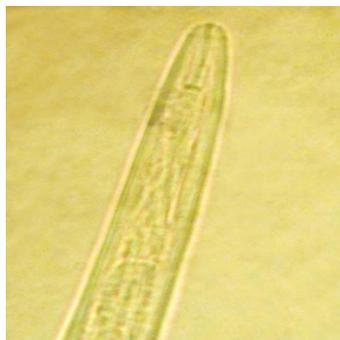
Jorhat



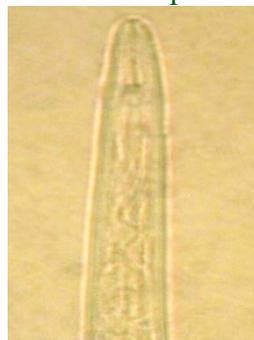
Kamrup



Kokrajhar



Lakhimpur



Nagaon



Sibsagar

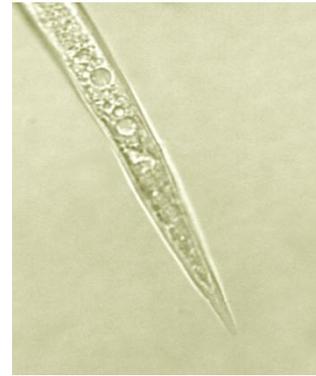
Fig.3 Tail of *M. incognita* J₂ from nine districts of Assam (40X)



Dhubri



Dibrugarh



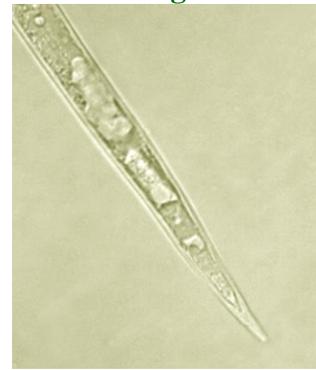
Golaghat



Jorhat



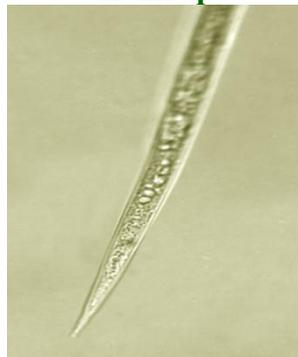
Kamrup



Kokrajhar



Lakhimpur



Nagaon



Sibsagar

Observations on perineal pattern of *M. incognita* collected from nine district of Assam exhibited same shape. Perineal patterns with high dorsal arch. Anus anteriorly located at 14-17 μm from the vulva. Striae distinct and wavy. Dorsal striae smooth and closely spaced. Similar finding were also observed by Chitwood (1949) and considered the perineal pattern as the most striking and distinguishing character for identification of root-knot nematode species. Kaur and Attri (2013) also opined that perineal pattern is the most stable characters of root-knot nematodes. Ley *et al.*, (1997) considered stylet length and tail length as the most stable characters in J_2 of *M. incognita*. Jepson (1987) also assessed the characters of different life stages like J_2 , female and male and concluded that tail length in J_2 , stylet length in female and male were most differential character whereas stylet length in juvenile was considered as supplementary differential character for identification of *Meloidogyne* species. Kaur and Attri (2013) stated that most of the morphometric characters of J_2 of *M. incognita* like body length, stylet length, head to median bulb length, distance from median bulb to excretory pore, tail length, anal body width, c and c' are highly variable character.

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