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Observations on the Foliar Nematode, *Aphelenchoides besseyi*, Infecting Tuberose and Rice in India

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Abstract: The foliar nematode *Aphelenchoides besseyi* causes white tip disease in rice (*Oryza sativa* L.) and floral malady in tuberose (*Polianthes tuberosa* L.). This nematode is widely distributed in the rice fields of many states of India, including West Bengal (WB), Andhra Pradesh (AP), Madhya Pradesh (MP) and Gujarat (GT). In order to generate information on intraspecific variations of *A. besseyi* as well as to confirm the identity of the nematode species infecting these important crops, morphological observation was undertaken of *A. besseyi* isolated from tuberose and rice from WB and rice from AP, MP and GT. The molecular study was only done for rice and tuberose populations from AP and WB. The variations were observed among the populations in the tail, esophageal and anterior regions, including the occurrence of four as well as six lateral lines in the lateral fields. The morphometrics of observed populations showed variations and those could be regarded as a consequence of host-induced or geographical variations. PCR amplification of the rDNA ITS 1 and 2 region of rice (AP) and tuberose (WB) populations of *A. besseyi* generated one fragment of approximately 830 bp, and the size of the ITS region was 788 bp and 791 bp for tuberose and rice population, respectively. Alignment of the two sequences showed almost 100% similarity. Blast analysis revealed a very high level of similarity of both the Indian strains to a Russian population. The Indian and Russian strains could be differentiated using restriction enzyme *Bccl*. Host tests revealed that rice (cv. IET 4094), oat (cv. OS-6) and teosinte (cv. TL-1) showed a typical distortion due to the infection of *A. besseyi*. Five germplasm lines of oat showed no infection of the nematode under field conditions. Local cultivars of onion, maize, chrysanthemum, gladiolus, and *Sorghum halepense* were also not infected by *A. besseyi*.

Key words: Aphelenchoides besseyi, diagnosis, distribution, host, India, morphology, rice, tuberose.

Amongst nematodes associated with rice (*Oryza sativa* L.) cultivation in West Bengal (WB), white tip nematode, *Aphelenchoides besseyi* is one of the most important due to its quarantine significance. Dastur (1936) reported its occurrence for the first time in rice from the former Central Provinces, now Chattisgarh region of Madhya Pradesh (MP), and later the nematode was reported widespread in India, particularly in the rice growing areas of Uttar Pradesh, MP, Tamil Nadu (Muniappan and Seshadri, 1964; Sivakumar, 1987), Gujarat (GT), Andhra Pradesh (AP) (Savitri et al., 1998) and Tripura (Nath et al., 1995). A serious outbreak of white tip disease was observed in 60% of the rice cultivars, with the most seriously affected cultivars being H.R.12 and Pankaj in AP (Jayaprakash and Joshi, 1979). The nematode causes yield losses of 10-15% in rice in GT (Thakar et al., 1987). The white tip disease of rice is severe in the southern and eastern states, where yield losses of 20% may occur (Prasad et al., 1986).

On tuberose (*Polianthes tuberosa* L.), the foliar nematode was first discovered to induce foliar disease in Hawaii (Holtzmann, 1968). Later, the occurrence of 'floral malady' caused by *A. besseyi* in tuberose was found in the Ranaghat areas of the Nadia district of WB (Chakraborti and Ghosh, 1993). Subsequently, *A. besseyi* was proven to be a pest of rice and tuberose in WB (Khan, 2001). The species was reported to cause a serious foliar disease of tuberose in the Mekong Delta of Vietnam, and based on morphological criteria and ribosomal RNA gene sequencing, the nematode was identified to be *A. besseyi* (Cuc and Pilon, 2007). Widespread occurrence of the white tip nematode infecting rice was reported from WB (Das and Khan, 2007, Khan and Das, 2009).

The widespread detection of white tip disease to rice in the southern and eastern states of India was reported and the foliar nematode problem is restricted to tuberose in WB and Odisha. Earlier studies were mostly on the occurrence of the problems and a detailed morphological and molecular evaluation of this nematode was thought to be essential for a better understanding of the species. Our preliminary light microscopic studies on the nematodes from these populations showed considerable variations in the body and stylet length including in the shape of tail, tail terminus and number of lateral lines. The objectives of this study were to evaluate populations of *A. besseyi* obtained from tuberose in WB and from rice in AP, MP, GT, and WB, using light and scanning electron microscopy (SEM) observations and molecular analysis and to assess the diagnostic value of morphological and molecular characters among the populations and to confirm the species infecting both rice and tuberose in India. Detailed morphometric and molecular observations of the nematode populations on rice and tuberose are presented herein.

Materials and Methods

Five populations of *A. besseyi* collected from rice (*Oryza sativa* L.) grains from Hyderabad, Andhra Pradesh

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(AP), Jabalpur, Madhya Pradesh (MP), Gujarat (GT), and West Bengal (WB) where *A. besseyi* populations infect both rice and tuberose, were studied for morphometric variations. Nematodes were extracted from rice grains after pounding with a mortar and pestle. The crushed grains were placed on single-ply tissues fitted over wire screens, which were then placed in Petri dishes containing tap water. The water level in the Petri dish was maintained to touch the bottom of the screen and tissue paper so that the nematodes could easily move down to the water below. Another Petri dish was placed on top of the screen and tissue paper to retard the evaporation of water. The entire assembly was kept for 15 to 20 hours at room temperature (30 to 35°C). *Aphelenchoides besseyi* from fresh, infected tuberose (*Polianthes tuberosa* L.) flower stalks obtained from foliar nematode-infested fields were extracted following the method of Khan and Pal (2001). Nematode specimens were killed in a hot-water bath, fixed in 3% formaldehyde and subsequently processed by the Seinhorst method (Seinhorst, 1959). The processed specimens were then mounted in anhydrous glycerine on glass slides. Photomicrographs of males, females and juveniles were taken with a Color Digital Camera (Retiga EXi, Q-Imaging, Austin, TX) attached to a compound microscope (a Leica Wild MPS48 Leitz DMRB, Leica Microsystems, Wetzlar, Germany). Altogether 24 parameters from at least 30 female of *A. besseyi* from each location and host in India were studied, as well as 30 males from WB on tuberose. The de-Man ratios and other values were determined from measurements taken with the help of an ocular micrometer (Hooper, 1986). For SEM, living specimens were fixed in 3% glutaraldehyde buffered with 0.05 M phosphate (pH 6.8), dehydrated in a graded series of ethanol, critical-point dried from liquid CO_2, and sputter coated with a 20 to 30-nm layer of gold-palladium.

Collection of nematode populations for DNA extraction: DNA was extracted from the populations of *A. besseyi* infesting tuberose in WB and rice in AP. Tuberose flower stalks showing distortions caused by foliar nematodes were cut and placed in sterile spring water in a sterile Petri plate (5 cm diameter). After about an hour, nematodes that had migrated to the water were transferred into 1.5 ml microcentrifuge tubes, pelleted and used for DNA extraction. Similarly, rice seeds collected from nematode-infested rice fields were placed in water and dissected to allow the nematodes to move into the water.

Extraction of nematode DNA: DNA was extracted from the above two populations separately according to the method described by Subbotin et al., (2000). Ten to fifteen µl of sterile distilled water was added into each tube containing nematodes, which were crushed with a microhomogenizer. About 50 µl of nematode lysis buffer (125 mM KCl, 25 mM Tris-HCl pH 8.3, 3.75 mM $MgCl_2$, 2.5 DTT, 1.125 % Tween 20, 0.025% gelatin) and 2 µl of proteinase K (600 g/ml) were added. The tubes were incubated at 65 °C (1h) and 95 °C (10 min) consecutively. Tubes were centrifuged at 16,000 g for 1 min and the supernatant containing DNA was stored at -20 °C until further use.

PCR amplification and cloning: Five µl of the DNA suspension was added to the PCR reaction mixture containing 0.2 mM dNTP′s, 0.2 µm of each primer (reverse and forward) and 2.5 µl 10 x Taq incubation buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 200 µM of each dNTP, 1 unit of Taq polymerase (Bangalore Genei, India), and double distilled water to a final volume of 25 µl. Primers (5′) TTGATTACG TCCCTGCCCTTT (3′) and (5′) TTCACTCGCCGTT ACTAAGG (3′) as described by Joyce et al. (1994) for amplification of ITS 1 and 2 were used in the PCR reaction. Amplification reactions were performed in an Eppendorf Gradient thermal cycler. Each cycle had denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 90 seconds; the cycle was repeated 44 times with a final extension of 72 °C for 5 min. Five-µl aliquots of amplification products were loaded on 1.2 % agarose gels prepared in 1X TAE (Tris-acetate-EDTA) buffer (pH 8.0), separated by electrophoresis (5 V/cm for 2.5 h) and stained with ethidium bromide. The gels were visualized using a gel documentation system (Vilber Lourmat, France). PCR products were purified with a PCR purification kit (Qiagen, Germany) as per manufacturer's protocols, and 2 µl of purified product was used for TA cloning using pGEM T vector (Promega, Madison, USA). Recombinant colonies were identified using an ampicillin and the blue white colony selection method according to the manufacturer's instructions, and were confirmed by PCR using the same primers used for PCR amplification. Plasmids were prepared and the inserts were sequenced and analyzed. Multiple sequence alignment was done by MUSCLE, alignment curation by Gblock, phylogenetic analysis by PhyML and tree construction by TreeDyne (Dereeper et al., 2008).

Host range tests: Onion (*Allium cepa* L. cv. Bombai red), chrysanthemum (*Chrysanthemum* sp.), gladiolus (*Gladiolus* sp. cv. Souvik), five germplasm of oat (*Avena sativa* L. cv. 0S-387, JO-03-309, JHO-2010-4, OL-1690, UPO-10-3), maize (*Zea mays* L. cv. Rajkumar), *Sorghum halepense* (L.) Pers.-Gramma-1, rice (*Oryza sativa* L. cv. IET 4094) and teosinte (*Euchlaena mexicana* Schrad. cv. TL-1) were grown in foliar nematode-infested plots at the Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Gayespur, WB. The onion, oat, maize, *Sorghum halepense*, gladiolus and rice were grown in a plot of 10 m x 1 m inserted between the heavily infested plots of tuberose infected by *A. besseyi*. The crops were grown during 2009-2011 in a standard spacing of the crops during their growing seasons. The teosinte was sown in rows in a plot of 5 m × 2.5 m with spacing of 40 cm between plants and 60 cm between rows. The teosinte plants (one of the three rows) were inoculated with tuberose foliar nematodes extracted from tuberose at 30, 45 and 60 days

TABLE 1. Morphometrics of female and male of *Aphelenchoides besseyi* infesting rice and tuberose in India[X], mean ± SE[Y], Range, Measurements in µm.

Characters	WB-tuberose (female~50)	AP-rice (female~37)	MP-rice (female~50)	WB-rice (female~30)	GT-rice (female ~30)	WB-tuberose (male~62)
Body length	642.04± 7.64 (514.00-784.00)	656.89± 8.87 (523.00-756.00)	651.12± 62.79 (522.00-775.00)	722.377±16.52 (520.00-840.00)	685.10±13.95 (543.00-845.00)	549.97±5.14 (455.00-635.00)
Body width	12.59±0.14 (11.30-15.00)	11.55±0.17 (9.75-13.40)	12.12± 1.18 (9.40-14.35)	14.51±0.18 (11.88-15.84)	12.95±0.28 (10.89-16.83)	11.36±0.06 (9.90-11.88)
Head-height	2.74±0.05 (2.50-3.40)	2.79±0.06 (2.43-3.48)	2.97-± 0.34 (2.37-4.95)	2.88±0.03 (2.47-3.47)	2.75±0.05 (2.37-3.46)	2.71±0.03 (2.27-3.46)
Head-width	5.66±0.61 (4.75-7.00)	5.50±0.09 (4.60-6.50)	6.02± 0.27 (5.44-6.73)	6.21±0.06 (5.54-6.93)	5.97±0.04 (5.74-6.44)	6.09±0.03 (5.44-6.73)
Stylet length	10.15±0.09 (9.82-10.78)	10.56±0.10 (9.35-11.90)	10.53± 0.51 (9.10-11.88)	10.59±0.10 (9.71-11.98)	10.63±0.14 (9.90-12.87)	10.20±0.07 (9.20-11.88)
Head to Centre of Median bulb(MB)	52.96±0.44 (45.9-61.20)	55.50±0.10 (46.7-62.05)	55.36± 3.42 (48.51-64.35)	58.77±0.52 (52.47-63.36)	60.30±0.88 (52.37-68.32)	50.78±0.53 (41.58-60.40)
Length of MB	11.96 ±0.13 (9.36-13.72)	11.37 ±0.19 (8.5-12.75)	11.53± 1.09 (8.91-13.46)	11.83±0.14 (9.90-12.87)	12.27±0.14 (10.89-13.86)	11.44±0.13 (9.40-13.86)
Width of MB	7.53±0.05 (6.80-8.82)	720±0.08 (6.5-7.50)	6.78± 0.61 (5.54-7.92)	8.10±0.10 (6.93-8.92)	7.34±0.23 (4.95-9.45)	7.93±0.08 (6.93-8.91)
Head-oes gland end	135.20±1.38, (110.00-158.00)	131.28±3.08, (111.60-148.3)	134.02± 8.87 (110.88-190.00)	151.97±2.52 (123.76-173.25)	141.88±2.76 (118.80-176.22)	133.64±1.45 (103.95-157.41)
Length of ant. Gonad/Testis	210.92±3.38 (196.30-299.20)	221.58±2.90 (183.6-248.60)	195.90±3.40 (139.52-233.64)	191.77±5.06 (149.49-267.30)	222.18±5.10 (184.14-275.00)	265.55±31.64 (200.00-306.00)
Length of PUS	33.94±0.47 (27.20-39.8)	34.84±0.75 (25.90-43.50)	34.54± 5.71 (24.75-46.53)	40.02±0.76 (32.64-52.47)	39.82±1.08 (33.66-52.47)	-
Length of vagina/spicule	6.45±0.10 (5.25-7.84)	5.70±0.10 (5.10-6.80)	6.15± 0.55 (5.44-7.43)	6.15±0.08 (5.44-6.93)	6.10±0.11 (4.95-6.93)	17.91±1.03 (15.84-19.80)
Head-excretory pore	67.09±0.76 (56.90-75.60)	67.72±1.25 (54.40-79.90)	67.04± 4.46 (56.43-77.22)	74.35±0.80 (63.36-83.16)	70.80±1.10 (60.40-83.16)	64.83±0.75 (49.50-78.21)
Tail Length	34.02±0.34 (29.7-37.24)	33.43±0.85 (27.20-42.50)	34.40± 2.80 (27.72-39.60)	37.76±0.59 (30.0-44.54)	36.03±0.70 (28.70-44.55)	33.76±0.40 (25.74-39.62)
Vulva position	452.77±4.61 (392.00-525.00)	472.39±8.65 (396.00-570.00)	458.58± 43.95 (370.00-555.00)	518.70±12.25 (300-650)	489.33±9.87 (390-595)	-
Anal body width(ABW)	8.77±0.11 (6.80-9.80)	9.00±0.14 (8.50-11.90)	7.56± 0.87 (5.74-9.90)	10.07±0.13 (8.91-11.48)	8.32±0.17 (6.73-9.90)	11.52±0.07 (9.90-12.67)
Junction of eso-intestine	61.61±0.94 (51.70-78.20)	61.37±0.60 (53.50-68.50)	69.61± 3.96 (62.37-82.17)	79.73±0.64 (72.26-85.14)	75.10±1.04 (65.24-88.18)	68.76±0.82 (51.97-81.18)
PUS/VBW(Vulval body width)	2.69±0.05 (2.16-3.52)	3.11±0.06 (2.41-3.96)	2.86±0.06 (1.92-4.2)	2.77±0.05 (2.20-3.54)	3.09±0.07 (2.52-4.27)	-
a	49.01±1.10 (40.5-60.75)	56.12±0.75 (46.15-64.75)	53.84± 3.66 (48.34-65.66)	49.76±0.89 (34.97-55.94)	53.16±0.87 (44.86-61.52)	47.78±0.42 (38.30-53.90)
b	11.63±0.16 (9.02-13.90)	11.17±0.16 (9.92-13.15)	9.35± 0.104 (7.75-11.22)	9.05±0.19 (7.20-11.18)	9.12±0.13, (7.12-10.68)	4.15±0.05 (3.63-5.96)
b′	4.77± 0.07 (3.67-6.57)	5.07± 0.10 (3.96-6.01)	4.89± 0.056, (3.68-6.03); 9.26	4.77±0.10 (3.57-5.95)	4.83±0.05, (4.22-5.26)	8.04±0.08 (6.90-9.80)
c	19.11± 0.24 (16.62-22.92)	19.27± 0.28 (16.67-21.60)	18.93± 1.04, (17.01-21.54); 5.50	19.14±0.34 (13.82-22.90)	19.05±0.24 (17.40-22.15)	16.38±0.18 (12.17-19.12)
c′	3.82± 0.05 (3.22-4.20)	3.72± 0.06 (3.00-4.20)	4.58± 0.40, (3.70-5.71); 8.84	3.75±0.04 (3.36-4.22)	4.35±0.07, (3.62-5.44)	2.93±0.03 (2.17-3.50)
V/T%	66.32-76.01 (70.52±0.26)	72.25±0.72 (66.60-79.90)	70.45± 1.44 , (65.08-73.08); 2.04	78.90±0.91 (57.69-84.96)	71.51±0.65 (63.24-79.66)	48.36±0.65 (35.93-60.00)

[X]WB= West Bengal, AP= Andhra Pradesh, MP= Madhya Pradesh, GT=Gujarat.
[Y]SE= Standard error of means.

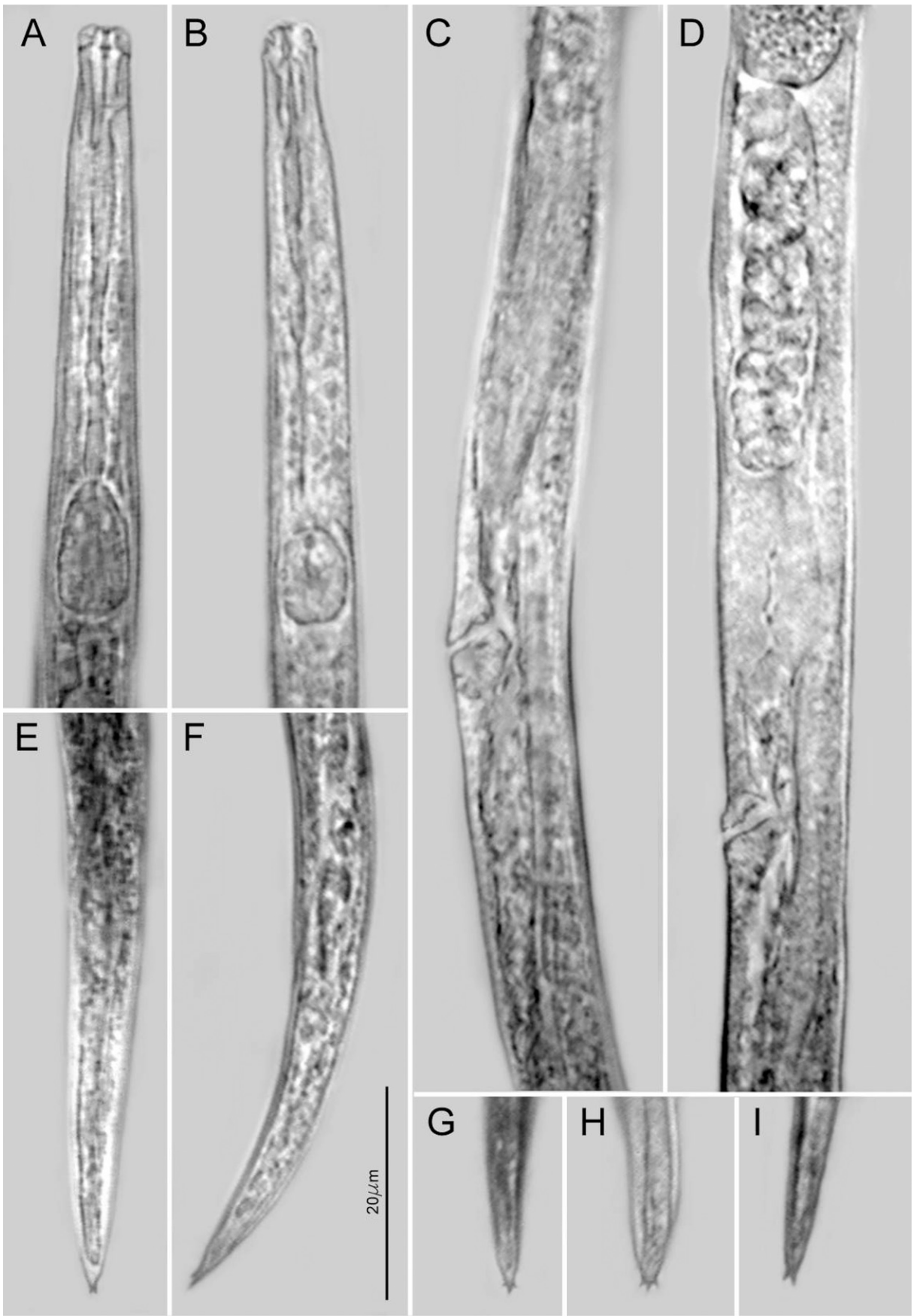

Fig. 1. Photomicrographs of *Aphelenchoides besseyi* female. A. Anterior end with esophagus; B-C: mid body region; E-F: Tail end; G-H-I: Tail tip, A-I: 20 µm scalebar.

after sowing at 500 nematodes/plant. After flowering, the plants were examined for infection by *A. besseyi* both in field and laboratory. Ten chrysanthemum plants were raised for two consecutive years (2009-2010) in pots and inoculated artificially by dispensing a nematode suspension of *A.besseyi* with a 5 ml dropper on the foliage and terminal points of 30 day-old cuttings at 2000 nematodes/plant. Infection by *A. besseyi* and development of symptoms on the plants were monitored regularly during the growing periods of the crops in both years. Five plant samples from ten sites within an experimental plot for each crop were collected and analysed in the laboratory for detection and estimation of nematode population. The observations on nematode population multiplication on the above crops were recorded from grains (100 grains) as well as from whole plants (at least five of onion and gladiolus) or plant parts (ten leaves including the growing points of chrysanthemum).

Results

Morphometrics and Morphology: Morphometrics of *A. besseyi* females and males are given in Table 1. The nematode body length varied from 514 μm to 845 μm in females. The body length indicated that it was moderately to highly variable in all the studied *A. besseyi* populations, whereas the ratios a, b′, b, c, and c′ were highly variable in the tuberose population. Stylet length (10-11μm), head to median bulb distance (HMB 53-61μm), and V% (71-79) were moderately to least variable. The mean length of the post-uterine sac (PUS) in *A. besseyi* populations from WB- rice and Gujarat-rice was relatively larger (40μm) than that of other populations (34-35μm) in this study. The mean b-ratio for MP-rice, WB-rice and GT-rice was less (9) than that of WB-tuberose (12) and AP-rice (11).

The *A. besseyi* populations studied in this investigation showed variations in the tail shape and terminal mucro and 2-3 processes, esophageal (shape of median bulb) and head end in the anterior regions, including the occurrence of four as well as six lateral lines in the lateral fields (Fig. 1-2).

PCR amplification of the rDNA ITS 1 and 2 region of each of the two studied populations of *A. besseyi* generated one fragment of approximately 830 bp. However, removal of primer sequences in the flanking 18S and 28S gave 788 and 791 bp ITS 1 and 2 sequences for rice and tuberose strains, respectively (Fig. 3). Further there was

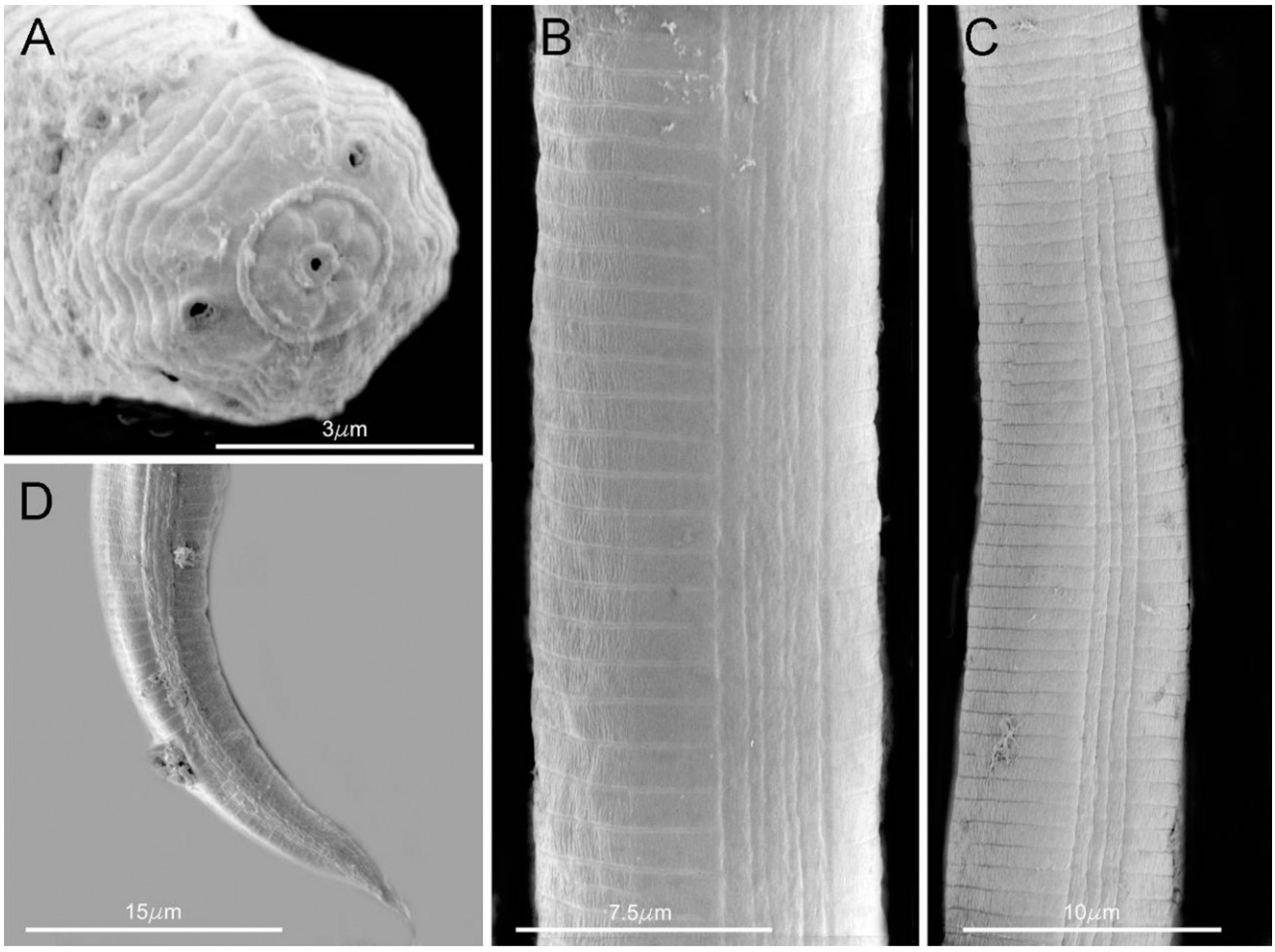

Fig. 2. SEM photomicrographs of A*phelenchoides besseyi* female. A. Head end; B-C: Lateral fields; D: Tail end.

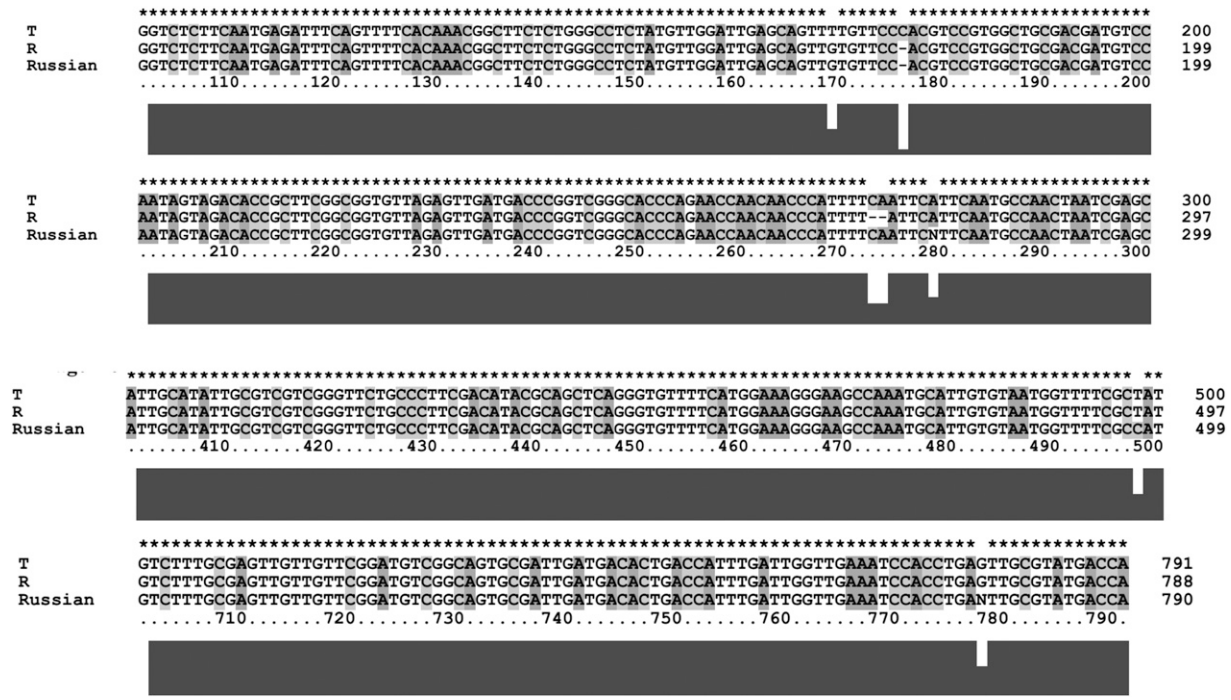

FIG. 3. Comparison of ITS 1 and 2 sequences of *Aphelenchoides besseyi* from India and Russia. Tuberose strain (T): rice strain (R) and Russian strain (Russian).

a thymine (T) instead of a guanine (G) at position 169 in case of the rice strain. Sequences of both populations have been deposited in GenBank under the accession numbers JF826519 (rice population) and JF826517 (tuberose population). The Indian populations are highly similar to a Russian population of *A. besseyi* (GenBank EU186069.1 790 bp). Multiple sequence alignment by Clustalw (Larkin et al., 2007) indicated a gap at position 176 in the sequence of Russian as in case of the tuberose strain. Interestingly, the thymine (T) at position 498 was replaced by cytosine (C) in the Russian population compared to both the sequences of the Indian strains. This sequence variation gave a restriction site for the enzyme *Bccl* only in the Russian population that could be used for differentiating it from the two Indian strains. The phylogenetic tree indicated that the two Indian populations and one Russian of *A. besseyi* were similar and distant from one population of *A. ritzamabosi* (Fig. 4).

Studies on crop hosts of *A. besseyi* (Table 2) revealed that the rice (cv. IET 4094), oat (cv. OS-6) and teosinte showed a typical distortion due to the infection of *A. besseyi*. Although, five germplasm lines of oat procured from the All India Coordinated Research Project on Forage Crops (Kalyani Centre, West Bengal) showed no infection of the nematode under field conditions. No infection of *A. besseyi* on local cultivars of onion, maize, chrysanthemum, gladiolus and *S. halepense* were recorded even on the few plants that showed some minor distortion like symptoms.

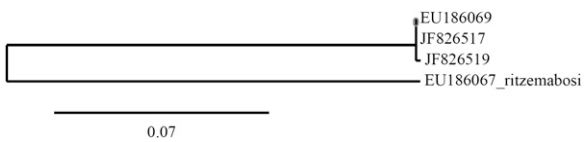

FIG. 4. Phylogentic tree among the three populations of *Aphelenchoides besseyi* and *Aphelenchoides ritzemabosi*.

DISCUSSION

In this study, morphometrics of *A. besseyi* populations from AP-rice, MP-rice, WB-rice and WB-tuberose, GT-rice conform with the measurements from the type host and locality (Christie, 1942), rice population from India (Dastur, 1936), Senegal (Fortuner, 1970), *Stylosanthes hamata* Taubert population (cultured on *Alternaria alternata*) from Queensland of Australia (Gokte et al., 1992), onion population from Sri Lanka (Lamberti et al., 1996) and rice population from Egypt (Amin, 2002). There were considerable variations in measurements among the populations from India, Senegal and Sri Lanka due to genetic variation and or host-induced variability. B'Chir (1977) observed that the population reared on *Impatiens balsamina* was longer than the ones reared on *Alternaria citri*. Rajan and Mathur (1990) proposed two groups of *A. besseyi* from India and Philippines isolates based on morphometry and culturability on fungal hosts and considered Cuttack (eastern India) and Pune (western India) populations belonged to one and Hyderabad (southern India) and Philippines isolates (Philippines) to another group. However, in the present

TABLE 2. Host tests with an *Aphelenchoides besseyi* population from tuberose.

Plant	Cultivar	Symptoms[z]	*A. besseyi* population/100grains	*A. besseyi* population/5 plants and/or 10 leaves
Onion (*Allium cepa* L.)	Local	-		0
Maize (*Zea mays* L.)	Local	-	0	
Oat (*Avena sativa* L.)	OS-6	+	7±0.33 (6-10)	
Teosinte (*Euchlaena mexicana* Schrad.)	TL-1	+	10±0.24 (8-14)	
Sorghum halepense (L.) Pers.	Local	-	0	
Gladiolus sp.	Souvik	-		0
Chrysanthemum sp.	Local	-		0
Rice (*Oryza sativa* L.)	IET-4094	+	17±0.32 (16-23)	

[z] +/- = symptoms appeared/not appeared.

study, no appreciable variation in morphometry of rice and tuberose populations of *A. besseyi* from India was noticed. The morphology of *A. besseyi* populations (rice and tuberose) showed limited variation in the tail shape, medial bulb of esophagus and head end of anterior regions. Furthermore, the occurrence of four as well as six lateral lines in the lateral fields of the rice population was observed in SEM as well as light microscope (LM) observations. Viewing the number of lateral lines with LM can be difficult in a nematode genus like *Aphelenchoides*. Hooper and Ibrahim (1994) also described *A. nechaleos*, a very closely related species of *A. besseyi* and viewed four lines with LM and six lines with SEM. *Aphelenchoides nechaleos* was also differentiated from 14 species of *Aphelenchoides* but the number of lateral lines was not considered as differentiating character.

Host tests indicated that the *A. besseyi* population from tuberose induces symptoms in oat (cv. OS-6), rice (cv. IET-4094), and teosinte (cv. TL-1) but not onion, maize, *S. halepense*, gladiolus and chrysanthemum. Lamberti et al., (1996) found *A. besseyi* inducing a distortion and twisting of stems and discoloration of the leaf apical portion on onion in Sri Lanka, and proved the casual relationship of the nematode population on stem tissues and disease symptoms and bulb yield loss. Over 67 different crops including maize, chrysanthemum and oat are attacked by *A. besseyi*, among them rice, strawberry and tuberose are the principal hosts in India (Khan, 2010). The *A. besseyi* population from tuberose was proven earlier to be the same population causing white tip disease of rice (Khan, 2001). However, in our study, maize, chrysanthemum and gladiolus were not infected, probably because of differential preference of the nematode population for specific hosts.

No genetic difference was found between the rice and tuberose strains of *A. besseyi*. Absence of the restriction site for *Bccl* in the ITS sequence of Indian strains could be used to differentiate the Indian strains and the Russian strains. Cuc and Pilon (2007) identified *A. besseyi* infection in tuberose from the Mekong Delta of Vietnam using morphological characters and partial sequences of two rRNA genes (small subunit 18S and large sub unit 28S). Their BLAST search of the GenBank database also showed homologous sequences of the 18S rRNA sequence with those of *Aphelenchoides ritzemabosi* (GenBank DQ901554) and *Aphelenchoides besseyi* isolate 98 from North Florida, USA (GenBank AY508035), with 602/628 (95%) and 595/643 (92%) nucleotide identities in homologous regions, respectively. Therefore, the present work provides both morphological and molecular sequence variations found in populations of *A. besseyi* infecting rice and tuberose that will enable researchers to identify the species infecting both rice and tuberose and to select DNA-cutting enzymes for use in molecular identifications tests that will be more rapid and accurate than conventional methods based on LM study. In addition, this study provides fundamental information that is needed for morphological and molecular diagnosis of this species to provide continued protection for the rice and tuberose industry in India and throughout the world.

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