

Short Communication:

The prevalence and species of root-knot nematode which infect on potato seed in Central Java, Indonesia

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Abstract. *Mutala'liah, Indarti S., Wibowo A. 2019. Short Communication: The prevalence and species of root-knot nematode which infect on potato seed in Central Java, Indonesia. Biodiversitas 20: 11-16.* Root-knot nematodes are considered as one of the most destructive pathogens of potatoes, especially on tuber seed. Infected potato seed will cause tuber malformation and the most important thing is as the main spreading source of *Meloidogyne* spp. The objective of this research was to know the prevalence and identify the species of root-knot nematode which attack the potato seed in four sub-districts of potato production centre in Central Java, Indonesia. Molecular and morphological identification was conducted for the nematode species identification. PCR assay using MIG primer to detect three tropical root-knot nematodes followed by sequencing was conducted for molecular detection, while the perennial pattern was conducted for morphological detection. Results showed that root-knot nematodes on potato seed were generally distributed in Central Java with the prevalence percentage between 14.28-88.23% on the three from four sampling area. The molecular and morphological identification show that species of root-knot nematodes identified on potato seeds were *Meloidogyne javanica*, *M. incognita*, and *M. arenaria*.

Keywords: Meloidogyne, potato, seed tuber, species

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are major pathogens of vegetables throughout the world and impacting both the quantity and quality of marketable yields, including on potato (*Solanum tuberosum*). The specific symptom of *Meloidogyne* spp. is root galls. It also cause another symptoms like chlorosis and stunted growth (Ye et al. 2015). *Meloidogyne* spp. is the most significantly impact on vegetable crops because it can be as a tuber and field borne pathogens (Coyne et al. 2018). *Meloidogyne* spp. are polyphagous nematodes that distributed worldwide. The highest impact is in the tropical region because of its favorable condition (Perry et al. 2009). In Indonesia, potato is one of the valuable horticultural commodities which is nowadays are attacked by *Meloidogyne* spp. The infection of this nematode on the potato tuber had also been reported in Argentina, Brazil, Japan, Libya, Zimbabwe, Saudi Arabia and Turkey (Vovlas et al. 2005).

Meloidogyne spp. can severely damage and cause a significant loss on potato depends on the nematode species (Vovlas et al. 2005). There were six species of *Meloidogyne*, i.e., *Meloidogyne chitwoodi*, *M. fallax*, *M. hapla*, *M. arenaria*, *M. incognita*, and *M. javanica*. In the colder climates, *M. chitwoodi*, *M. fallax* and *M. hapla* are causing a major problem, whereas *M. arenaria*, *M. incognita*, and *M. javanica* are in the warmer climates (Vovlas et al. 2005; Onkendi and Moleleki 2013). *M. incognita* is the most commonly found and causes loss until

80% (Devi and Kumari 2014), while in Brazil, *M. javanica* was the major species which found in infected potato at about 97.8% (Medina et al. 2017). In Tasmania, Australia, *M. hapla* and *M. fallax* caused serious problem on potato tuber. However, the most significant damage was caused by *M. fallax* (Hay et al. 2016) Another report of infectious potato tuber in Belgium, Ireland, Netherlands, Portugal, United Kingdom, Chile, and United states was caused by *M. minor* (Wesemael et al. 2014). In Asia, especially South China, some species of *Meloidogyne* (*M. incognita*, *M. enterolobii* and *M. javanica* not only caused significant losses on potato tuber, but also have serious problem on ornamental plant and fruit trees (Hu et al. 2011).

Meloidogyne spp.' infection on potato tuber in Indonesia was first reported in Ngablak, Magelang, Central Java in 2014. Almost 97% of fields were infested by *Meloidogyne* spp. (Suri and Jayasinghe 2014). Infected potato tuber caused some galls in the surface of the tuber. Females of *Meloidogyne* spp. with transparent color appeared on the flesh of tuber. It may cause blisters form on the tuber and make it unmarketable. More importantly, the infected seed tuber can facilitate this nematode's dissemination into new areas (Vovlas et al. 2005).

Central Java, Indonesia is the center of potato plantation. However, there was no report either about infectious tuber caused by *Meloidogyne* spp. or the species of this nematode. Therefore, the objective of this research was to examine the prevalence of *Meloidogyne* spp. that attacked potato seed and identify the species diversity of

Meloidogyne spp. in the potato production area in Central Java, Indonesia.

MATERIALS AND METHODS

Root-knot nematode distribution

Survey was conducted in four potato producing areas in Central Java, i.e., Ngablak Sub-district (Magelang District), Kledung Sub-district (Temanggung District), Kejajar Sub-district (Wonosobo District), and Pejawaran Sub-district (Banjarnegara District) from January until May 2015. Cluster sampling technique was carried out to determine the exact amount of the farmer's warehouse samples from each sub-district. The total number of farmers in four sub-district was 1935, consisted of 1250 farmers in Kejajar (Personal communication with field officer 2015), 335 farmers in Ngablak (Personal communication with field officer 2015), 200 farmers in Pejawaran (Personal communication with field officer 2015), and 150 farmers in Kledung (Personal communication with agriculture institution 2015). The total number of sample was determined by the population and precision (10%) which was 95 farmers or 5% from each sub-district, consisted of 17 farmers in Ngablak, 8 farmers in Kledung, 60 farmers in Kejajar and 10 farmers in Pejawaran. The data were analyzed according to the prevalence of nematodes. The frequency of the nematode was determined from the relationship between the numbers of sample where the nematode observed then divided by the total number of samples taken, and multiplied by 100 to express as the percentage of the prevalence (Sawadogo et al. 2009).

Molecular identification

Sampling for species identification was taken by purposive sampling technique from each sub-district. Only the potato which infected by *Meloidogyne* spp. would proceed for further species identification. CTAB method (Subandiyah 2003) was used for DNA extraction using 30 females of *Meloidogyne* spp. from each area then examined by PCR. The PCR program for MIG primer (Table 1) was 2 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 54°C as the annealing temperature and 1 min at 72°C (Zijlstra et al. 2000). Samples for the sequencing process was sent to Bioscience Singapore, then analyzed by Bioedit program to matched the DNA sample with DNA data in Gen Bank. Phylogenetic tree was made by neighbor-joining method (MEGA 6.06) to determine the similarity of each sample with the species of *Meloidogyne* that was listed in GenBank.

Morphological identification

Morphological identification for *Meloidogyne* spp. was based on the perennial pattern of female's posterior. Perennial pattern was the proper manner for morphological detection on *Meloidogyne* spp. because it is easy to obtain from the infected plant and it tends to be stable from the environmental changes (Eisenbeck 1985). Five female nematodes were collected from each sub-district for singly identification. This step was carried out to observe the existence of other minor species that could not be amplified in the molecular identification. Female *Meloidogyne* spp. was isolated from the infected tuber and placed on the slide glass under a stereoscopic microscope. An incision was made by using a scalpel in the middle of the female body to cut the cuticle into half equatorially. The posterior region consisting of the perennial pattern was carefully cut off and trimmed. The perennial pattern was gently brushed using alcohol to remove any attached debris (Singh 2009). The perennial pattern was captured by Optilab which was connected to the microscope Olympus BH2 by 400 times magnification.

RESULTS AND DISCUSSION

The prevalence of *Meloidogyne* spp.

Meloidogyne spp. was considered as one of the most impact yield losses both quality and quantity of the postharvest product, especially on potato. Potato tuber which was infected by *Meloidogyne* spp. had malformation on the outer surface of tuber (Figure 1.A) caused by some galls that released from nematode's infection. The appearance of the tuber was worst so it would be decreasing the quality of tuber and make tuber unmarketable. On the other hand, it was descending the postharvest quantity because the galls inhibit nutrition and water uptake, so that potato tuber was poorly developed. Infected potato tuber caused by *Meloidogyne* spp. was obtained from each sub-district in Central Java. The female nematode and egg mass were shown in the flesh of tuber. An egg mass of *Meloidogyne* spp. usually stick on the posterior region of female nematodes with the brownish color that appeared on the flesh of tuber (Figure 1.B). The female nematode was a little bit difficult to see because it had the same color with the flesh of tuber. The female nematode usually looks grouping on the flesh of tuber but sometimes it also looks singly (Figure 1.C).

Table 1. Primer identification of *Meloidogyne* spp.

Species	Primer code	Sekuen 5'-3'	DNA target (bp)	Source
<i>Meloidogyne incognita</i> ,	MIGF	ACACAGGGGAAAGTTTGCCA	500	Qiu et al. (2006)
<i>M. javanica</i> , <i>M. arenaria</i>	MIGR	GAGTAAGGCGAAGCATATCC		

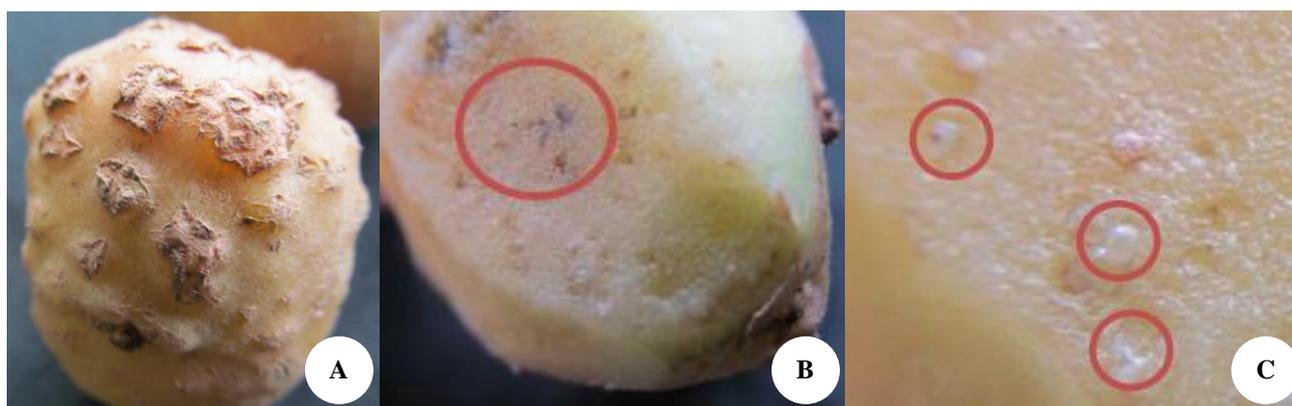


Figure 1. A. Potato seed malformation was caused by *Meloidogyne* spp. B. Egg mass of *Meloidogyne* spp., C. Female of *Meloidogyne* spp.

Table 2. Prevalence percentage of infected potato seed in Central Java, Indonesia

Sub-district	District	Farmer's warehouse samples	Number of infectious sample	%
Ngablak	Magelang	17	15	88.23
Kledung	Temanggung	8	5	62.50
Kejajar	Wonosobo	35	5	14.28
Pejawaran	Banjarnegara	10	0	0
Total		70	25	35.71

The survey results showed that *Meloidogyne* spp.' infection only presented in three sub-districts: Ngablak, Kledung, and Kejajar. Meanwhile, infectious potato by *Meloidogyne* spp. was not found in Pejawaran. The highest percentage of prevalence was in Ngablak (88.23%) then followed by Kledung (62.50%) and the least presence was in Kejajar which account for 14.28% (Table 2). The number of total sample in Kejajar was reduced because from 60 farmers, there were only 35 farmers that had potatoes in their warehouse at the sampling time.

The incidence of *Meloidogyne* spp. infection was influenced by the geographical factors of each area. Ngablak is located in Magelang District at 1293 meter above sea level (m asl) (Pekab Magelang 2015), with temperature of 20-27°C and the soil type is andosols (Pemda Ngablak 2015). Kledung is located in Temanggung District at 1138 m asl, the temperature 20-30°C and the soil type is andosols (Pemkab Temanggung 2015). Kejajar is located in Wonosobo District at 1378-2250 m asl, the temperature 12-21°C in the afternoon and 5-9°C in the night. The soil type of this district is andosols (Pemkab Wonosobo 2015). Pejawaran is located in Banjarnegara District at more than 1700 m asl (Taher et al. 2012). The temperature and the soil type in Pejawaran are the same with Kejajar (NAWASIS 2010).

Based on that geographic information data, the infection incidence of *Meloidogyne* spp. was found in the area between 1100-1300 m asl and which also related to the

optimum temperature for *Meloidogyne* spp. The temperature in Ngablak and Kledungs is suitable for *Meloidogyne* spp.'s development. Therefore the percentage of infection incidence was very high. In Kejajar, distribution infection only found in 5 areas located approximately at 1300 m asl, but it was not found in some areas above 1300 m asl. Likewise in Pejawaran, the infection incidence of *Meloidogyne* spp. on potato tuber was not found. It was caused by its location as the highest between all observed sampling areas, so that the temperatures also lower than others. *Meloidogyne* spp. cannot well develop in the cold temperature. The optimum development temperature for *M. javanica* and *M. arenaria* was 28°C, whereas *M. incognita* was 25 or 30°C. In the lowest temperature, *M. javanica* failed to reproduce egg (Bridge and Starr 2007; Perry et al. 2009).

In addition, another factor that influenced the infection incidence was the soil type. All of the surveyed areas had andosols soil type. Andosols soil type is volcanic soil type that good for agriculture. Besides that, andosols also have good aeration (Bowman and Arts 2001), thus it was easier for nematode migration. Nematode migration was decreased when the clay proportion of soil was up to 30% (Prot 1977), whereas in the sandy soil type, nematode can migrate until 75 cm for about nine days (Netscher and Sikora 1990). It was also in line with Archidona-Yuste et al. (2018), *M. incognita* has a high positive correlation with sandy loam soils, whereas it has a negative correlation with silt and clay (Asif et al. 2015). Sandy soil texture can facilitate J2 to penetrate the roots, thus the gall and egg mass were significantly increase in this soil type (Kim et al. 2017). Furthermore, potato infection caused by *Meloidogyne* spp. had high intensity in dry seasons harvesting (Private communication), whereas this research was taken on rainy season harvesting. Considering the percentage of infection incidence result in Central Java on rainy seasons harvesting, it could increase if the survey is taken in dry seasons harvesting. This result was also confirmed by Karajeh and Mohawesh (2016), root galling index and nematode population was higher at 80 and 100% irrigated than 20, 40 and 60%.

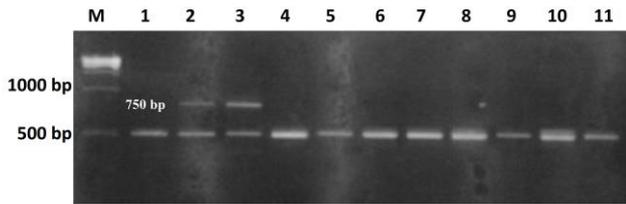


Figure 2. Amplification band used MIGF/MIGR primer. M: 1 kb DNA Ladder (Vivantis), Samples (1-11), 1-4: Ngablak samples, 5-7: Kejajar samples, 8-11: Kledung sample

Molecular identification

MIG primer was used for molecular identification of three species of *Meloidogyne* i.e., *Meloidogyne incognita*, *M. Javanica*, and *M. arenaria*. Qiu et al. (2006) had shown that MIG primer would detect *M. incognita*, *M. javanica* and *M. arenaria* on 500 bp. All samples produced amplicons on 500 bp; however, sample number 2 and 3 (sample from Ngablak) also amplified another band, i.e., 750 bp (Figure 2). This was a new result appeared on the visualization of PCR using MIG primer that should be only appeared on 500 bp.

The molecular identification using MIG primer on this research showed a variation on the amplification products with the study of Qiu et al. (2006). There were two types of amplicons, i.e., 500 bp and 750 bp, whereas in a study by Qiu et al. (2006), the MIG primer amplified and produced only a single band on 500 bp for the three species of *Meloidogyne* spp. (*M. incognita*, *M. javanica*, and *M. arenaria*). The different result might be caused by the different PCR program (Zijlstra et al. 2000) used on this study. Therefore, the DNA band showed the differences.

The sequencing results can identify the nematodes species of the amplicons products. The phylogenetic tree (Figure 3) showed that the sample amplicons on 500 bp from all samples was closely related with *M. javanica* with the bootstrap value: 94. Samples on 750 bp were closely related with *M. incognita* with the bootstrap value: 83.

From this analysis, it can be inferred that only one species of *Meloidogyne* found in Kejajar and Kledung, i.e., *M. javanica*. On the other hand, there were two species of *Meloidogyne* found in Ngablak, i.e., *M. javanica* and *M. incognita*.

Morphological identification

Morphological identification of *Meloidogyne* spp. was conducted using the perennial pattern on five female nematodes in each sub-district. Morphological identification was used for supporting the molecular identification. Morphological identification of *Meloidogyne* spp. was difficult to distinguish because their perennial pattern look like similar (Oliveira et al. 2011). Based on the perennial pattern, there were two species of *Meloidogyne* found in Ngablak, i.e. *M. javanica* (Figure 4.A) and *M. incognita* (Figure 4.B). Both Kejajar and Kledung were found only one different species of *Meloidogyne*. In Kejajar, the *Meloidogyne* species was *M. arenaria* (Figure 4.C), whereas in Kledung was *M. incognita* (Figure 4.D-E), which were different to the molecular analysis results. The differences of species in morphological and molecular identification might be due to only the dominant species that appeared on molecular identification. Thus the morphological identification was carried out to identify the minor species singly.

The parameters used in the morphological identification were the shape of perennial pattern and the cuticle layer (striae). *M. incognita* has dorsally arch high and slim (Taher et al. 2012). Striae were very wavy and tight. *M. javanica* has oval till pear-shaped, smooth and less wavy of striae (Williams 1972), and have two lateral line as the key to distinguish with another species of *Meloidogyne* (Taher et al. 2012). The lateral lines divide the dorsal and ventral striae (Kaur et al. 2016). *M. arenaria* has oval till round shaped, and flat striae (Williams 1972). Dorsally arch short and slim (Taher et al. 2012). Both *M. arenaria* and *M. javanica* have low dorsal arch, whereas *M. incognita* has high dorsal arch (Aydinli and Mennan 2016).

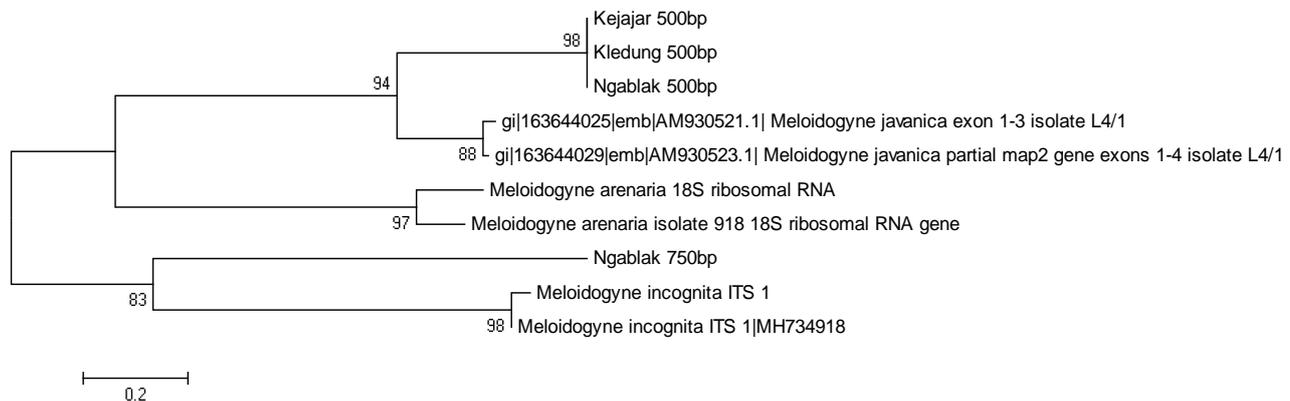


Figure 3. Phylogenetic tree of *Meloidogyne* spp. with neighbor joining method (MEGA 6.06)

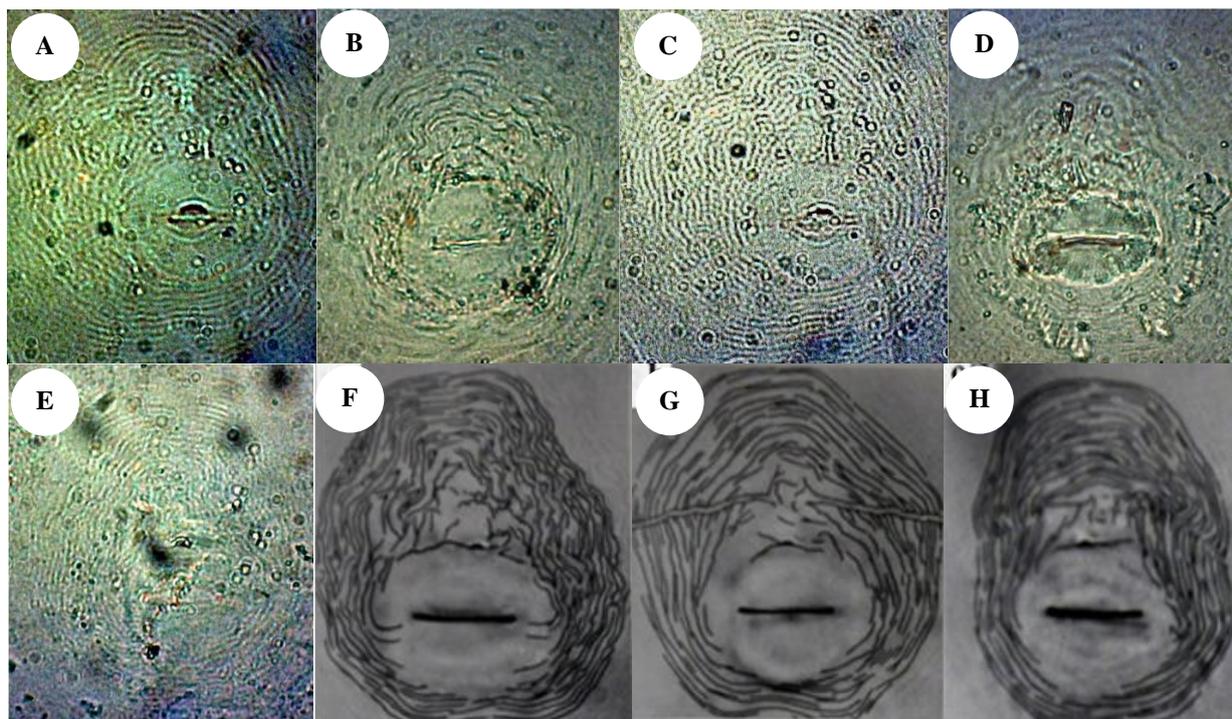


Figure 4. Perennial pattern of *Meloidogyne* spp.; A. Ngablak sample 1 (*M. javanica*), B. Ngablak sample 2 (*M. incognita*), C. Keajar sample (*M. arenaria*), D-E. Kledung (*M. incognita*), F-H. *M. incognita*, *M. javanica*, *M. arenaria* (E-F: Eisenbeck et al. 1981)

In conclusions, the results of this survey showed that the prevalence infection was generally distributed in Central Java (35.71%), dispersed in Ngablak (88.23%), Kledung (62.5%) and Keajar (14.28%). Species of *Meloidogyne* spp. which infect the potato center producing area in Central Java were *M. incognita*, *M. javanica*, and *M. arenaria*. These three species were the most destructive root-knot nematode in the tropical area. The *Meloidogyne* species found in this study are the common *Meloidogyne* spp. in the temperate region. This information is important to anticipate the wide distribution of the tuber infection and for nematode management strategy. The use of infectious tuber seed on the plantation should be prohibited moreover on the dry season harvesting. This was also in line with Evlice and Baryam (2016), conclude that unqualified potato seed tuber was forbidden to use in uninfected fields and must be followed by other management strategy to prevent the damage on potato tuber.

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