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First Report on the Root Knot Nematodes *Meloidogyne* spp. of Sweetpotatoes in Sorong Regency, West Papua-Indonesia

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Abstract

Sweet potatoes with abnormal tubers were found in Salawati District, Sorong Regency, West Papua Province. Surveys in the period of April to September 2014 found the disease symptoms include tubers fail to grow and turn into a hard roots, smaller size tubers and number of tubers per hill decreased. Length of infected tubers were 3 to 8 cm (compare to healthy tubers 15 to 18 cm), overgrown roots and developed knot on the tubers surface and roots surrounded by necrotic areas. Staining method and root disection were succesfully found Meloidogyne spp. adult females inside the sweet potato roots and tubers. Two species of root knot nematodes that were M. incognita and M. javanica were identified based on morphology of female perineal pattern. Using PCR technique, two specific primers of Meloidogyne species MI-F / MI-R and Fjav / Rjav were successfully amplifed 999 bp and 720 bp DNA fragments which were related to M. incognita and M. javanica, respectively.

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Phylogenetic tree analysis showed that M. incognita and M. javanica of West Papua were closely related with M. incognita and M. javanica from China and Malaysia. This paper is the first report of an association of Meloidogyne spp. with the decreasing sweet potato production in West Papua.

Keywords: abnormal tuber, Meloidogyne incognita, Meloidogyne javanica.

1. Introduction

Sweet potato is one of the crops that are important for the people of Papua and West Papua. Aside from being a staple food, sweet potato is considered as a heritage that plays an important role in various ceremonies, religious, social communication and a source of family income. Sweet potato become one of the leading commodity locally in the province of West Papua with the development area covers several counties. Sorong Regency is one of the centers of development of sweet potato in the province of West Papua (West Papua Provincial Agriculture Office, unpublished). Based on observations in the field, root knot nematode *Meloidogyne* spp allegedly caused the decreasing quality and quantity of sweet potato tubers. *Meloidogyne* spp. have been reported in association and cause damage to sweet potato in some countries for instance Nigeria. In this country *M.incognita* led to declining yields of sweet potato varieties Puerto Rico, White Star and Red Nancy [16]. On the island of Kyushu Japan, as many as 96% of sweet potato plantation were infected by parsitic nematode *M. incognita*, *M. arenaria* and *M. javanica* [9]. This study was aimed to describe disease symptoms as well as to detect and identify the Meloidogyne species on sweet potato in Sorong, West Papua Province.

2. Materials and Methods

2.1. Sampling and Nematode Extraction

Symptomatic plants were sampled from Salawati District Sorong Regency, West Papua Province in April 2014. Extraction of nematodes and preparation of semi-permanent slides were carried out in the Nematology Laboratory, Department of Plant Protection, Bogor Agricultural University. Nematode morphological and molecular identification were conducted in the Molecular Laboratory of Applied Research Institute of Agricultural Quarantine in Bekasi-Indonesia. Tubers and root samples were selected from 3 plantations which during harvest. Plant samples (a total of 285 plants) were randomly selected. An 100 plants were sampled for further laboratory examination. Samples were wrapped in dampened newspaper and stored in a cool box [3].

2.2. Morphological Identification

Morphological identification was initiated with observation of the nematodes presence inside the infected roots and tubers with acid fuchsin staining method [2, 23].Females of root-knot nematodes recoverred from infected roots and tubers were used as the subject for morphological identification. The nematodes were removed from roots and tubers that were previously soaked in water for 24 hours in order to be more lenient. The female nematodes were dissected and prepared for morphological identification based on the perineal pattern. Perennial

pattern was observed using a light microscope with a magnification of 400 times, followed by species identification according to Eisenback [4].

2.3 Molecular Identification

Nematode DNA extraction was done by using FastDNA Spin Kit lysing matrix A (MPBio) by means of extraction Super FastPrep-1 (MPBio). PCR reactions were performed using Phusion High-Fidelity PCR Master Mix (Thermo) in 96-well Verity machine (Applied Biosystems). PCR reaction was using specific primers for M. incognita that were MI-F (forward primer) with the composition of sequences 5'-GTG CAG AGG ATT CCCAG TCT-3' and MI-R (reverse primer) with the composition of sequences 5'-ACG AGG CTT CTC AAC ATA CGT CC-3' [1]. Amplification of M. javanica was using specific primers Fjav (forward primer) 5'-GGT CGA GCG TTG AAC TGA GC-3'dan Rjav (reverse primer) 5'-CAG CAG CTT GCC TGG TAT AAC AC-3 '[28].

PCR reaction was using TaqTm Dream Green PCR Master Mix (2x) (Thermo Scientific), with DNA amplification process includes an initial denaturation at a temperature of 94º C for 4 min, denaturation at 94º C for 30 seconds, annealing *M. incognita* at 62º C for 30 seconds and *M. javanica* at 55 º C for 45 seconds, and extension process at 72º C for 1 min. The number of cycles were 45 cycles for *M. incognita* and 35 cycles for *M. javanica* and followed by a final extension at 72º C for 7 min. DNA amplification products were separated on 1.2% agarose gel dissolved in 1x TAE and added PeqGreen as much as 4-6 ml / 100 ml agarose. Measurement of DNA fragments was using a 1 kb DNA ladder marker (Fermentas, United States of America). Electrophoresis was performed at a voltage of 70 volts for 50 minutes and visualized using UV Transilluminator (Darkhod 1000).

DNA sequencing was performed using pairs of specific primer *M. incognita* and M. javanica in 1st BASE Science Genetics. Sequencing results were analyzed using the program of basic local alignment search tool (BLAST) with the optimization program to obtain DNA base sequences in the NCBI website. Sample alignment was done by ClustelW (Bioedit) and phylogenetic analysis was conducted by MEGA5 softwares.

3. Results and Discussion

3.1 Disease Symptom

Observation of 285 plant samples found 253 plants were diseased and 32 were healthy plants. Disease symptom observed indicated the difference between the symptoms appeared on red and white varieties of sweet potato. Symptoms of white sweet potato tuber was an abnormal growth, tuber failed to grow and turn into a hard root, tuber surface overgrown root (hairy roots) and tuber size was smaller. Length of diseased tuber was 3 to 8 cm, compared with healthy tuber size was 15 to 18 cm. The number of tubers in each clump decreased (Figure. 1b). The number of tubers produced by infected plants were generally only 1, while the healthy plants were able to develop 3 to 4 tubers (Figure 1a).

Meloidogyne incognita was reported to cause yield losses and a significant decline in the quality of sweet potato varieties of Jasper, Gold rush, and Porto Rico [22]. Olabiyi in [16] reported that *M. incognita* cause yield loss

Puerto rico sweet potato varieties of 1963 kg / ha, White star of 1814 kg / ha and Red nancy 1755 kg / ha compared with the yields of the uninfested agricultural land. Infection of *M. incognita* on sweet potato caused tuber production was reduced by 47.7% [5], and the susceptible cultivar (Kayode, TIS 70 357-op-1-791, and TIS 4400-2) decreased 72.3% to 83.2 % [18]. Yield losses due to *Meloidogyne* spp infection in the Philippines reached 50% to 100% on infected land planted with sweet potato continuously for three seasons [6,7]. Solomon sweet potato varieties infected with *Meloidogyne* spp. produced abnormal tuber with irregular shape compared with Antiqua varieties that are more resistant to *Meloidogyne* spp. [20].

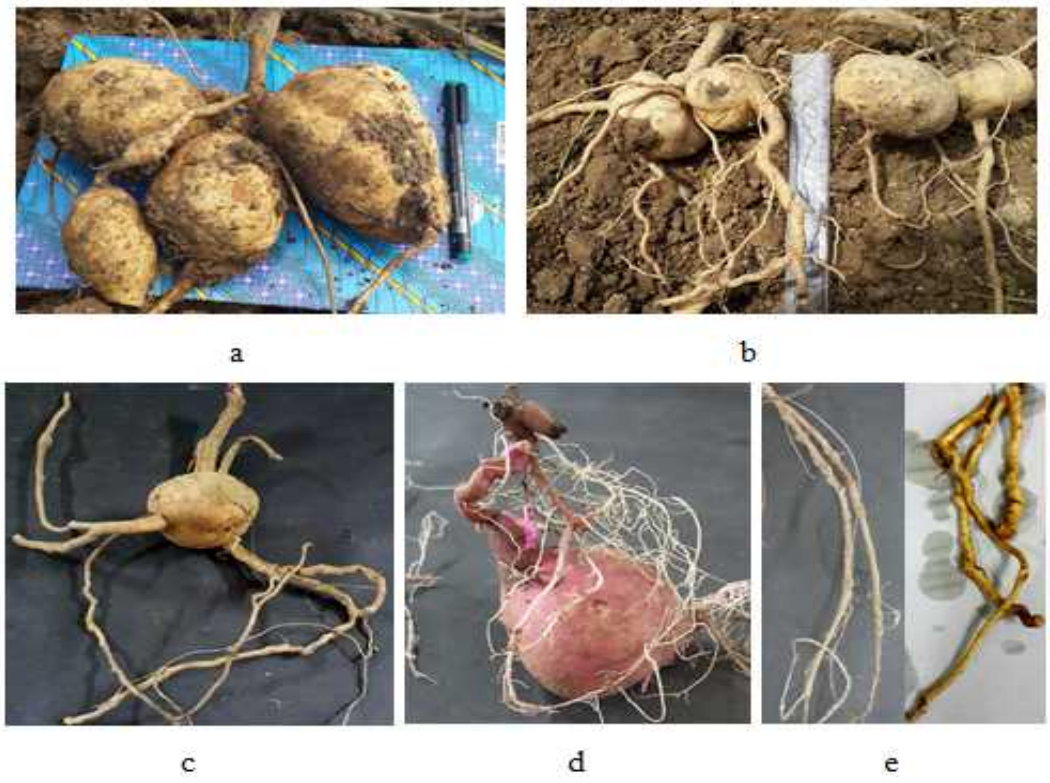

Figure 2: Disease symptom of *Meloidogyne* spp. on sweet potato in Sorong, (a) healthy sweet potato (b)the roots smaller tuber size, (c) grown roots on the tuber surface, (d) the hairy roots, and (e) gall and necrotic on sweet potato roots.

Other symptoms on infected tubers and roots are the formation of knots along the roots surrounding by necrotic spot. The knot formed on the tuber has a smaller size than ones on other host plants. *Meloidogyne* spp. is not able to induce the formation of prominent gall in sweet potato tubers [13]. Infection of *Meloidogyne* spp. the red sweet potato has led to a smaller size with root fibers grow at the base and tip of tubers (Figure. 2d). Root knots were developed on these root fibers. Infection of *Meloidogyne* spp. at the beginning of plant growth led to the formation of gall on the root surface [12, 27] and sometimes on the tuber so that the root has a rough texture [27].

3.2 Meloidogyne species based on the Morphological Observation

Egg mass and female of *Meloidogyne* were observed inside the infected sweet potato roots by nematode staining and direct disection methods (Figure 3). The vermiform second stage juvenile of *Meloidogyne* penetrate root tissue, growing and changing its body shape become pyriform with rounded posterior. *Meloidogyne* is a sedentary endoparasitic with the anterior part of the female nematode is located near the root stele while the posterior part is in the cortex. Adult females produce eggs that are agregated in gelatin matrix at 30 days after entering the root [17]. Acid fuchsin staining method was succesfully detect the presence of egg masses within the root tissue and root surface. Something similar was reported by Osunlola and Fawole (2015b), egg masses were laid in the root cortical cells as well as near the root surface [19].

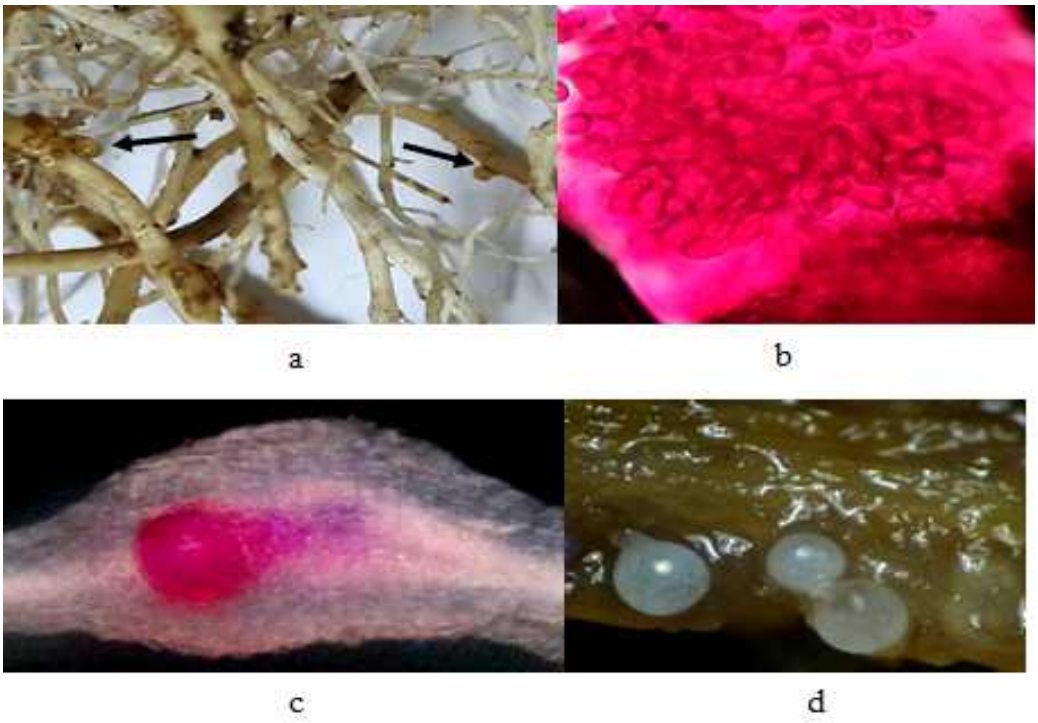

Figure 3: Root knot nematodes in the infected roots: (a) eggs mass on the root surface, (b) stained egg mass, (c) stained adult female inside the roots of red sweet potato (d), female nematode on dissected sweet potato root tissue

The field observation showed plants with the similar symptoms spread across the sweet potato plantation in Salawati District, Sorong Regency. Two species of *Meloidogyne*, that were *M. incognita* and *M. javanica* were identified based on the characteristics of female perineal pattern [4]. *M. incognita* is characterized by a high and narrow dorsal curved, while in the outer part is slightly dilated and somewhat flat, does not have lateral lines and clearly visible stria. *M. javanica* is characterized by two lateral lines that separate very clearly the dorsal from the ventral curved [4].

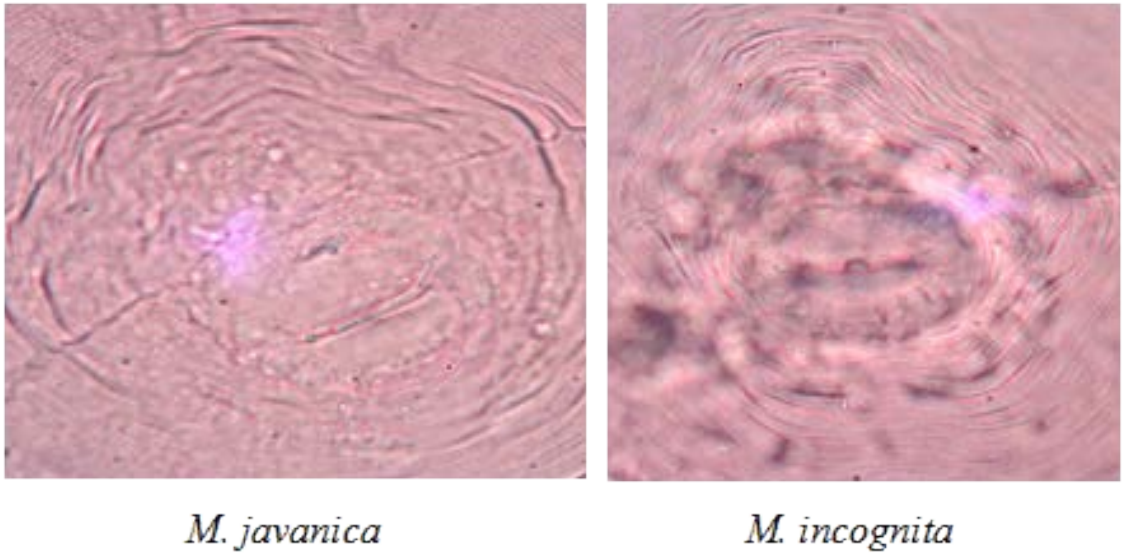

Figure 4: Perineal pattern morphology of *M. javanica* and *M. incognita*

3.3 Meloidogyne Species based on Molecular Identification

PCR technique using specific primer of *M. incognita* was amplified DNA fragment of 999 bp for all sample tested including eggs and females of *Meloidogyne* from Sorong Regency. Similar method was applied using specific primer of *M. javanica* and resulted the DNA fragment of 720 bp. these results were in-line with morphological identification confirming that *M. incognita* and *M. javanica* were the causal pathogen of decreasing sweet potato in Sorong Regency, West papua.

Further investigation using the BLAST analysis showed that *Meloidogyne* species of West Papua have high similarity with the species *M. incognita* and *M. javanica* from China and Malaysia with the homology level of 95% and 92%, respectively. Phylogenetic analysis showed that *M. incognita* of West Papuan categorizes into one group with *M. incognita* isolates from Malaysia and China. Similar result was shown for *M. javanica* which falls into one group with isolates from China and Malaysia (Figure 6). This results indicate that *M. incognita* and *M. javanica* of West Papuan are closely related to *M. incognita* and *M. javanica* from China and Malaysia.

Meloidogyne incognita is an important pathogen of sweetpotato in tropical and subtropical regions with very broad distribution and host range [13]. *M. incognita* in Indonesia have been identified in various types of plants such as tomato [26], patchouli [14], Ginger [15], soybean [21], carrots in Cipanas West Java [10,8], and Central Java [24] . *M. javanica* root knot nematodes are widespread and becoming important pathogens in tropical regions [11]. In Indonrsia nematodes have been reported to infect tomato plants [25], patchouli [14] and carrot [8], while the sweet potato has not been reported. This paper is the first report on sweet potato disease caused by *M. incognita* and *M. javanica* in Sorong, West Papua.

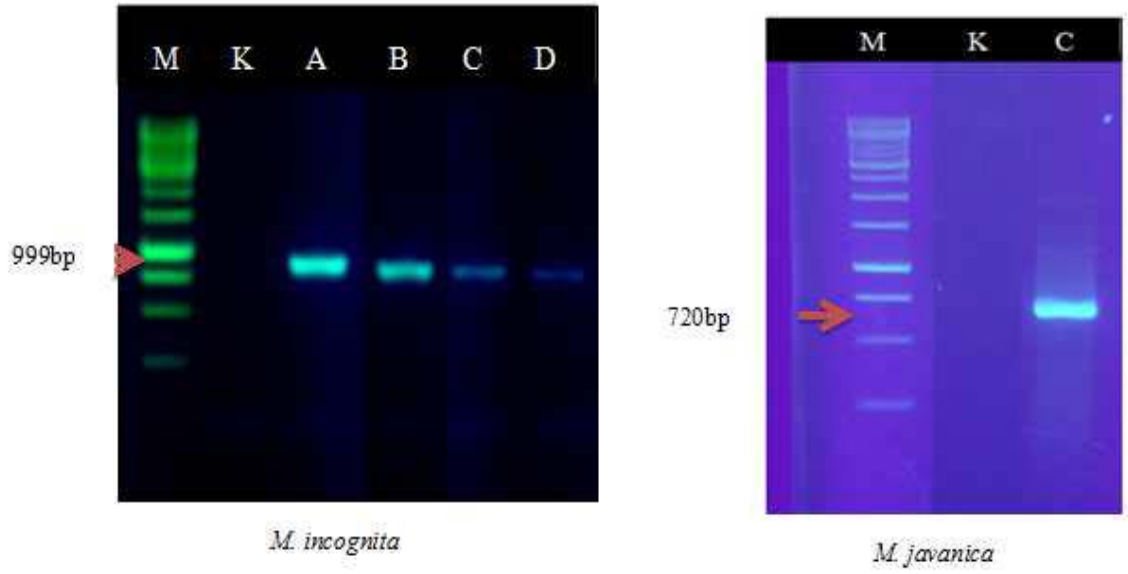

Figure 5: Amplified DNA fragments of *Meloidogyne* spp from Sorong, West Papua on a 1.2% agarose gel electrophoresis: *M. incognita* (999 bp) and *M. javanica* (720 bp), 1 kb DNA marker (M); control (K); 20 adult females (A); 5 egg group (B); 10 Group of eggs (C); 0.1 g of root knot (D).

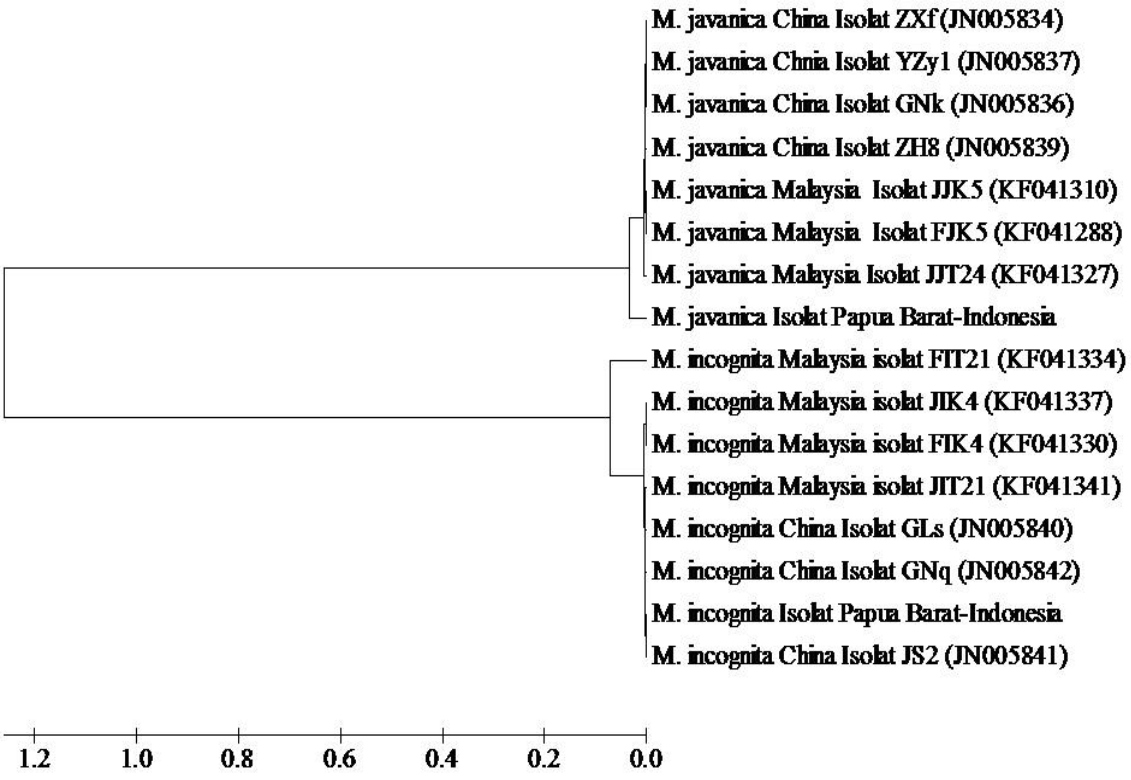

Figure 6: Phylogenetic tree of *M. incognita* and *M. javanica* of sweet potato in Sorong, West Papua

4. Conclusion

The declining sweet potato plants in Sorong Regency, West Papua was caused by the root-knot nematode Meloidogyne spp. Two Meloidogyne species, that were *M. incognita* and *M. javanica*, were idenfied based on their morphological and molecular characteristics. Both species were closely related with *M. incognita* and *M. javanica* from China and Malaysia.

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