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Identification of *Aphelenchoides* Species in Local Varieties Rice Seeds of North Sumatra Based on Molecular Markers

Identifikasi Spesies *Aphelenchoides* pada Benih Padi Varietas Lokal Sumatera Utara Berdasarkan Marka Molekuler

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ABSTRACT

The nematode *Aphelenchoides besseyi* was successfully detected and morphologically identified in the rice seeds of Samosir local varieties, namely 'Siserang', 'Sipining', 'Sibandung', and 'Saratus Ari'. This study aimed to confirm the identity of *Aphelenchoides* species using universal primers as markers for the D2-D3 expansion region of the 28S rDNA gene. Nematodes were extracted from the seeds of the North Sumatra local varieties including 'Siserang', 'Sipining', 'Sipala', 'Sirias', 'Sigambiri Merah', and 'Sigambiri Putih'. Nematodes from the commercial rice variety 'Pak Tiwi-1' was used for comparison. DNA extraction of *A. besseyi* nematodes was carried out using the Holterman method. The amplification process used universal primers D2A-D3B, producing a DNA fragment of approximately 750 bp. DNA amplification of *A. besseyi* from the Samosir, Simalungun, and Pakpak Bharat Districts successfully produced DNA fragments of approximately 750 bp. Nematodes obtained from the Siserang, Sigambiri Merah, Sirias, Sigambiri Putih, Sipala, and Sipining varieties showed 99.8%–100% homology with *A. besseyi* isolates from the Indonesia commercial rice variety, Japan, Brazil, and China.

Keywords: amplification, homology, nematode, rDNA

ABSTRAK

Nematoda *Aphelenchoides besseyi* berhasil dideteksi dan diidentifikasi secara morfologi pada benih padi varietas lokal Samosir, yaitu 'Siserang', 'Sipining', 'Saratus Ari', dan 'Sibandung'. Penelitian ini bertujuan untuk mengonfirmasi identitas spesies *Aphelenchoides* dengan menggunakan primer universal sebagai penanda untuk daerah ekspansi D2-D3 gen 28S rDNA. Nematoda diekstraksi dari benih padi varietas lokal Sumatera Utara yaitu 'Siserang', 'Sipining', 'Sipala', 'Sirias', 'Sigambiri Merah', dan 'Sigambiri Putih'. Nematoda dari varietas padi komersial 'Pak Tiwi-1' digunakan sebagai pembanding. Ekstraksi DNA nematoda *A. besseyi* dilakukan dengan menggunakan metode Holterman. Proses amplifikasi menggunakan primer universal D2A-D3B, menghasilkan pita DNA sekitar 750 pb. Amplifikasi DNA *A. besseyi* dari Kabupaten Samosir, Simalungun, dan Pakpak Bharat berhasil menghasilkan fragmen DNA sekitar 750 pb. Nematoda yang diperoleh dari varietas 'Siserang', 'Sigambiri Merah', 'Sirias', 'Sigambiri Putih', 'Sipala', dan 'Sipining' menunjukkan homologi 99.8%–100% dengan isolat *A. besseyi* yang berasal dari varietas padi komersial Indonesia, Jepang, Brasil, dan Cina.

Kata kunci: ampifikasi, homologi, nematoda, rDNA

INTRODUCTION

The nematode *Aphelenchoides besseyi* is one of the rice plants most important parasitic nematodes. The only method for *A. besseyi* to spread quickly is through seed infection. The nematodes invade the rice panicle and multiply with a generation time of 10 days at 25 °C (Diana *et al*. 2018). This nematode can survive in rice seeds for 2-3 years. The viability of *A. besseyi* is relatively stable during storage and can cause white tip symptoms in seedlings (Purnamasari 2018).

The most striking symptom caused by this nematode is the appearance of chlorotic spots on the tips of newly growing leaves as they emerge from the leaf sheath. These leaf tips then dry out and shrivel, while the rest of the leaf often appears unaffected. Young leaves on infected seedlings may exhibit a characteristic pattern of white spots or chlorotic spots. Although the leaf edges may become distorted and wrinkled, the leaf sheath usually shows no visible symptoms (OEPP/EPPO 2017). This nematode can also infect rice grains and cause brown spots on rice grains (Siregar 2024). The nematode *A. besseyi* has been reported to be distributed almost throughout Indonesia and is an important plant-disrupting organism in rice plants (Kementan 2020).

The transmission of *A. besseyi* through rice seeds can facilitate its rapid dissemination among rice fields throughout Indonesia. This nematode species has been detected in several rice-producing regions of North Sumatra, including Deli Serdang Regency (Percut Sei Tuan, Pagar Merbau, Sunggal), Batubara Regency (Medang Deras, Talawi, Sei Balai), Simalungun (Hutabayu Raja, Pematang Bandar, Siantar Marimbun), Langkat (Stabat, North Securai, Gebang), and Serdang Bedagai (Sei Bamban, Dolok Masihul, Teluk Mengkudu) (Suswati *et al*. 2025).

Hutauruk (2018) reported the presence of *A. besseyi* in commercial varieties such as 'Ciherang', 'Inpari 30', 'Inpari 32', 'Inpari Sidenuk', and 'Mekongga'. The presence of *A. besseyi* was also successfully detected based on morphological and morphometric characters in local varieties in Samosir Regency, North Sumatra. These local varieties include 'Siserang', 'Sipining', 'Sibandung', and 'Saratus Ari' (Sitanggang *et al.* 2024).

Molecular approaches can confirm and provide a more precise and efficient way to distinguish among *Aphelenchoides* species. Molecular identification is carried out by polymerase chain reaction (PCR) using universal primers. Molecular identification can be done to confirm the accuracy of morphological and morphometric identification results. This study aimed to confirm the identity of *Aphelenchoides* species using universal primers as markers for the D2-D3 expansion region of the 28S rDNA gene.

MATERIALS AND METHODS

Sampling of Rice Seeds

Random sampling was conducted by collecting 5 kg of rice seed samples placed in plastic bags and labeled accordingly. The seeds were obtained from farmer-based seed multiplication activities. The seed sources originated from Samosir Regency (Pangururan and Palipi Districts), Simalungun Regency (Dolok Silau and Raya Districts), and Pakpak Bharat Regency (Salak and Kerajaan Districts) (Table 1). The local rice varieties collected included 'Siserang', 'Sipining', 'Sipala', 'Sirias', 'Sigambiri Merah', and 'Sigambiri Putih'. These local varieties were compared with a commercial rice variety, 'Pak Tiwi-1'.

Extraction of *Aphelenchoides besseyi* Nematode

According to the International Seed Testing Association (ISTA) standard (2014), rice seed samples weighing up to 10 g (approximately 400 seeds) were taken, excluding the hilum. Nematodes were extracted from the rice seeds using a modified Baermann funnel technique (Remeeus and Pelazza 2014). The rice seed segments were placed on a gauze mesh suspended in a container filled with water, allowing the nematodes to migrate to the bottom of the container. The samples were kept in a dark place at room temperature for 24 hours. After soaking, the seed fragments were filtered using a 400 mesh sieve.

Molecular Identification of Nematodes

DNA extraction of *A. besseyi* nematodes was carried out based on the method of Holterman *et al*. (2006). Five nematodes were placed in a 0.2 mL (PCR collection tube) filled with 25 µL of nuclease-free water. A total of 25 µL of extraction buffer solution (810 µL of nuclease-free water, 40 µL of 200 mM NaCl, 100 µL of 200 mM Tris-HCl pH 8, 10 µL of 1% β-mercaptoethanol, and 40 µL of 800 µg mL^{-1} proteinase K) was added to the PCR tube. The mixture was homogenized for 1 minute and then incubated in a water bath at 65 °C for 90 minutes and 99 °C for 5 minutes. Nematode DNA is ready for amplification or storage at -20 °C.

Table 1 Rice seed sampling locations

Regency	District	Varieties
Samosir	Palipi	Sipining
	Pangururan	Siserang
Simalungun	Raya	Sigambiri Merah
	Dolok Silau	Sigambiri Putih
Pakpak Bharat	Kerajaan	Sirias
	Salak	Sipala
		Pak Tiwi-1

Amplification using universal primers (D2A-D3B). The DNA band was 750 bp in size with the forward primer sequence D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and the reverse sequence D3B (5'-TCG GAA GGA ACC AGC TAC TA-3'). Each PCR reaction consisted of 12.5 µL of 2× MyTaq™ HS Red Mix (Thermo Scientific, US), 1 µL of 10 µM forward primer, 1 µL of 10 µM reverse primer, 2 µL of DNA template, and 8.5 µL of nuclease-free water, resulting in a total volume of 25 µL. The PCR amplification program consists of a predenaturation stage at 95 °C for 3 minutes, denaturation at 95 °C for 30 seconds, annealing (primer attachment) at 54 °C for 30 seconds, elongation (synthesis of new DNA strands) at 72 °C for 15 seconds, and a post-elongation stage at 72 °C for 10 minutes. The cycles are repeated 30 times (Kurniawati 2024).

Visualization of DNA bands is done through electrophoresis. PCR products successfully amplified were then sent to 1st Base (Selangor, Malaysia) for DNA sequencing. Sequencing results were analyzed using the basic local alignment search tool (BLAST) program. Nucleotide sequences were analyzed using ClustalW multiple alignment on Bioedit Sequence Alignment Editor software version 7.2.5. Evolutionary relationships between isolates were constructed using Molecular Evolutionary Genetic Analysis software version 11.0 (MEGA11) with a bootstrap of 1000 replicates.

RESULTS

Identity of *Aphelenchoides besseyi* based on Universal Primers

PCR amplification of *A. besseyi* from Samosir, Simalungun, and Pakpak Bharat Districts showed success in obtaining DNA fragment with a size of ±750 bp using primer pairs D2A and D3B (Figure 1). The genes in these primers have been successfully used to distinguish species of nematode. Amplification results confirmed that *A. besseyi* was found in all varieties tested. The nucleotide alignment of *A. besseyi* was compared using *A. besseyi* nematode isolates from different countries (Table 2).

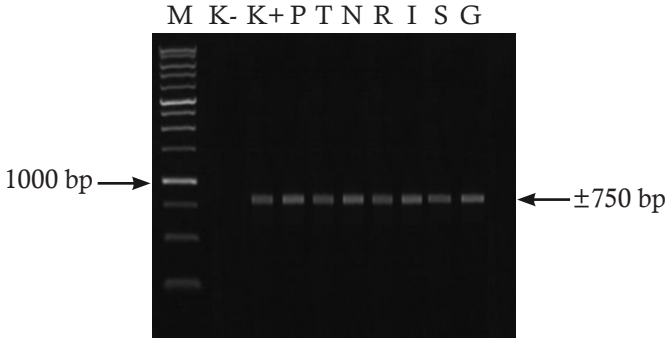

Figure 1 Amplification results of the 28S-rDNA region of *Aphelenchoides besseyi* measuring 750 bp. M, 1 kb DNA ladder marker (Thermoscientific, US; K-, negative control; K+, positive control; P, Pangururan; T, Indonesia; N, Raya; R, Kerajaan; I, Palipi; S, Salak; and G, Dolok Silau.

Table 2 Homology level of Indonesian *Aphelenchoides besseyi* isolates from North Sumatra to isolates from several countries based on 28S-rDNA nucleotide sequencing using Bioedit program version 7.2.5

Accession number	Origin of the isolate	Homology (%)*										
		1	2	3	4	5	6	7	8	9	10	11
PV158080.1	Indonesia	-										
PV158078.1	Indonesia (Salak)	100	-									
PV158079.1	Indonesia (Pangururan)	99.8	99.8	-								
PV158077.1	Indonesia (Kerajaan)	99.8	99.8	100	-							
PV158081.1	Indonesia (Raya)	99.8	99.8	100	100	-						
PV158082.1	Indonesia (Palipi)	100	100	99.8	99.8	99.8	-					
PV158083.1	Indonesia (Dolok Silau)	99.8	99.8	100	100	100	99.8	-				
KX356774.1	China	99.7	99.7	99.8	99.8	99.8	99.7	99.8	-			
KT692700.1	Brazil	99.8	99.8	100	100	100	99.8	100	99.8	-		
KT692690.1	Japan	99.5	99.7	99.7	99.7	99.5	99.7	99.5	99.7	-		
KX356837.1	Netherlands	83.7	83.7	83.8	83.8	83.8	83.7	83.8	83.7	83.8	83.7	-

*1, *Aphelenchoides besseyi* Indonesia (PV158080.1); 2, *A. besseyi* Salak (PV158078.1); 3, *A. besseyi* Pangururan (PV158079.1); 4, *A. besseyi* Kerajaan (PV158077.1); 5, *A. besseyi* Raya (PV158081.1); 6, *A. besseyi* Palipi (PV158082.1); 7, *A. besseyi* Dolok Silau (PV158083.1); 8, *A. besseyi* China (KX356774.1); 9, *A. besseyi* Brazil (KT692700.1); 10, *A. besseyi* Japan (KT692690.1); and 11, *A. ritzemabosi* Netherlands (KX356837.1)

Sequencing results showed that *A. besseyi* isolates from local and commercial varieties in Indonesia were closely related and clustered with *A. besseyi* isolates from Brazil, China, and Japan. Significant homology suggests that the nematodes belong to the same species. The *A. ritzemabosi* isolate as an outgroup has a homology percentage of 83.7 with other *A. besseyi* isolates. The close homology among these isolates suggests significant genetic similarity, reflecting a common evolutionary origin (Figure 2 and Figure 3).

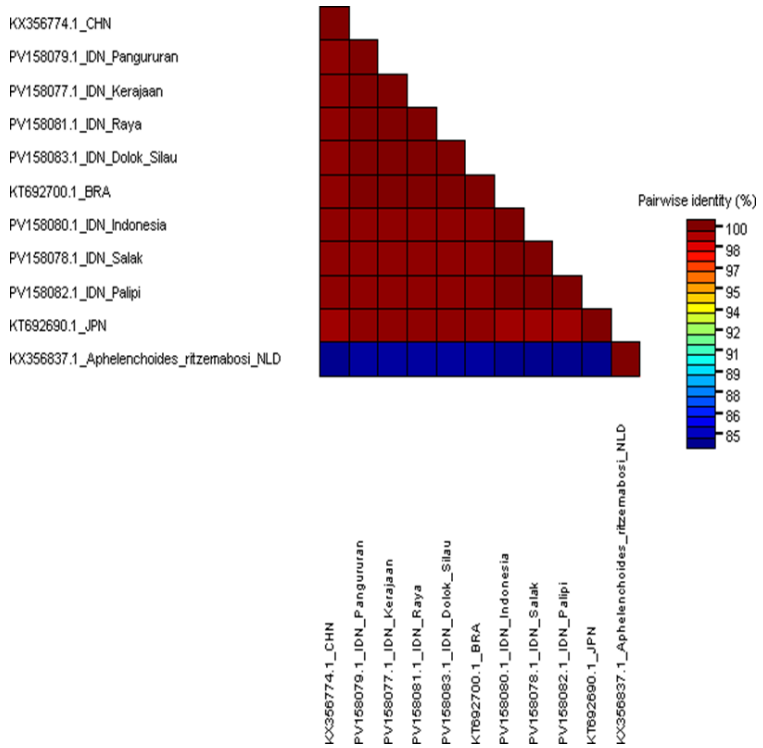

Figure 2 Homology level of Indonesian *Aphelenchoides besseyi* isolates from North Sumatra to isolates from several countries based on 28S-rDNA nucleotide sequencing using sequence dermacation tool.

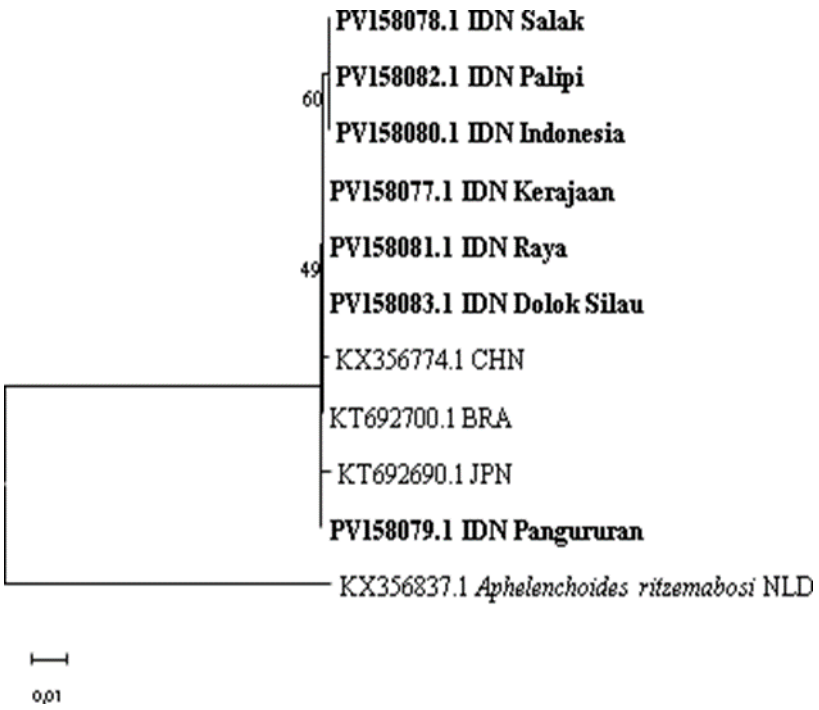

Figure 3 Phylogenetic tree of Indonesian *Aphelenchoides besseyi* isolates from North Sumatra against isolates from several countries based on 28S-rDNA nucleotide sequencing. Phylogenetic analysis was performed using the Neighbor-Joining method with 1000 bootstrap replicates. Nematodes *Aphelenchoides ritzemabosi* outgroup was used as outgroups.

DISCUSSION

The ability of *A. besseyi* to survive on seeds means that *A. besseyi* inoculum is already present before planting, thus creating a high risk of white tip disease incidence in the field. The number of *A. besseyi* carried on the seeds will determine the severity of the disease in the field (Diana *et al*. 2018).

'Sigambiri Merah', 'Sigambiri Putih', 'Sirias', and 'Sipala' are dryland rice varieties, whereas 'Siserang' and 'Sipining' are wetland rice varieties. The 'Pak Tiwi-1' rice type served as a reference variety alongside local varieties. The type is an inbred rice that withstands waterlogging, rendering it appropriate for cultivating *A. besseyi* (Rahman *et al.* 2018).

Phylogenetic analysis of nematodes utilizes nucleotide sequence data to trace the origin of seed-borne nematodes (Sembiring *et al.* 2019). Nucleotide alignment analysis confirmed that the nematodes detected were *A. besseyi. Aphelenchoides besseyi* nematodes obtained from 'Siserang', 'Sigambiri Merah', 'Sirias', 'Sigambiri Putih', 'Sipala', and 'Sipining' varieties had a homology level of 99.8%–100% with *A. besseyi* isolates from the commercial rice variety in Indonesia, Japan, Brazil, and China. The close homology among these isolates suggests significant genetic similarity, reflecting a common evolutionary origin. This study also confirmed the importance of geographical factors in spreading genetic diversity in nematodes (Kurniawati 2024). A high homology level indicates that the nematodes are the same species (Rahman *et al*. 2018).

The white tip nematode from local rice seed varieties in North Sumatra, namely 'Siserang', 'Sigambiri Merah', 'Sirias', 'Sipining', 'Sipala', and 'Sigambiri Putih', was confirmed as *A. besseyi*, which has homology level of 99.8%–100% with *A. besseyi* isolates from Japan, Brazil, and China.

REFERENCES

Diana DR, Supramana, Mutaqin KH, Kurniawati F. 2018. Distribusi nematoda pucuk putih padi *Aphelenchoides besseyi* di Pulau Jawa. Jurnal Fitopatologi Indonesia 14(4):129–137. DOI: https://doi.org/10.14692/jfi.14.4.129.

Holterman M, Wurff VDA, Elsen VDS, Megan VH, Bongers T, Holovachov O, Bakker J, Helder J. 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Molecular Biology and Evolution. 23(9):1792–800. DOI: https://doi.org/10.1093/molbev/msl044.

Hutauruk MY. 2018. Survei dan karakterisasi biologi *Aphelenchoides besseyi* di Sumatera Utara [skripsi]. Bogor (ID): Institut Pertanian Bogor.

[ISTA] International Seed Testing Association. 2014. *Detection of Aphelenchoides besseyi on Oryza sativa.* Annexe to Chapter 7: Seed Health Methods: 7-025-2. Bassersdorf (CH): ISTA.

[Kementan] Kementerian Pertanian. 2020. Peraturan Menteri Pertanian Republik Indonesia Nomor 25 Tahun 2020 tentang Jenis Organisme Pengganggu Tumbuhan Karantina. Jakarta (ID): Kementan

Kurniawati F. 2024. Kajian nematoda daun *Aphelenchoides* spp. di Pulau Jawa: distribusi dan identifikasi, teknik perbanyakan, dan pengendalian [disertasi]. Bogor (ID): Institut Pertanian Bogor.

[OEPP/EPPO] Organisation Européene et Méditerranéene pour la Protection des Plantes/ European and Mediterranean Plant Protection Organization. 2017. PM 7/39 (2) *Aphelenchoides besseyi*. Bulletin OEPP/EPPO 47(3):384–400.

Purnamasari R. 2018. Pengaruh lama penyimpanan benih terhadap populasi nematoda *Aphelenchoides besseyi* Christie dan vigor bibit padi [skripsi]. Bogor (ID): Institut Pertanian Bogor.

Rahman RM, Munif A, Kurniawati F. 2018. Deteksi dan identifikasi nematoda *Aphelenchoides besseyi* dari benih padi. Jurnal Fitopatologi Indonesia. 14(2):39–46. DOI: https://doi.org/10.14692/jfi.14.2.39.

Remeeus PM1, Pelazza N. 2014. Detection of *Aphelenchoides besseyi* on *Oryza sativa*. Di dalam: Kaufman B, El-Khadem R, Muschick P, Taylor J, editor. *International Rules of Seed Testing*. Bassersdorf (CH): ISTA.

Sembiring AIM, Kurniawati F, Supramana. 2019. Karakterisasi internal transcribed spacer (ITS) rDNA nematoda pucuk putih (*Aphelenchoides besseyi* Christie). Jurnal Agroekoteknologi. 12(1): 16–25. DOI: https://doi.org/10.21107/agrovigor.v12i1.5085.

Siregar KM. 2024. Keragaman nematoda parasit tanaman padi sawah di Kelompok Tani Sri Asih Desa Tanjung Rejo, Kecamatan Percut Sei Tuan, Kabupaten Deli Serdang [skripsi]. Medan (ID): Universitas Medan Area.

Sitanggang D, Supramana, Kurniawati F. 2024. Keberadaan nematoda *Aphelenchoides besseyi* pada benih padi varietas lokal di Kabupaten Samosir, Sumatera Utara. Jurnal Fitopatologi Indonesia. 20(5):226–233. DOI: https://doi.org/10.14692/jfi.20.5.226-233.

Suswati S, Azwana A, Candra IA, Kurniawati F, Siregar MK. 2025. Morphological, molecular, and morphometric analysis of *Aphelenchoides besseyi* on organic rice (*Oryza sativa*) seeds in the Beringin District, Deli Serdang Regency, Indonesia. Makara Journal of Science. 29(1):158–168. DOI: https://doi.org/10.7454/mss.v29i1.2335.