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Endophytic fungi diversity of Kalimantan Siam 11 local rice cultivar

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Rice plant form mutualistic symbiosis with endophytic fungi. The endophytic fungi have important role in improving growth and health of the the rice plant as a host. The role of endophytic fungi in local rice cultivar in Indonesia has not been studied well. Therefore, this research aimed to study diversity of the endophytic fungi associate with the Kalimantan Siam 11 local rice cultivar. The fungi were isolated from all parts of mature plant consisting of roots, stems, leaves, brown rice, spikelets, and husks; germinated seeds which consists of radicles and celeoptiles; and seedling which consists of primary roots and shoots using the surface sterilization method. Fungi identification used combined morphological and molecular characteristics. Morphological identification carried out using colony and microscophic features, while moleculer identification used DNA sequences of ITS1-5.8S-ITS2 rDNA region with ITS1 and ITS4 primers and continued with phylogenetic analysis. A total of 13 species were isolated and identified from all part of the rice plant organs. The identified fungi were *Cladosporium oxysporum, C. coloradense, Poaceascoma lochii, Penicillium brefeldianum, P. citrinum, Talaromyces pinophilus, T. angelicus, T. macrosporus, Simplicillium obclavacum, Fusarium humuli,* and *F. keratoplasticum* strain 1 and 2, *Pseudopestalotiopsis simithheae* and *Sarocladium oryzae*. All parts of the rice plant organs inhabited by endophytic fungi except germinated seed and seedling primary roots. The highest fungi diversity was found in the leaves whereas the lowest diversity was observed in the husks.

Keywords: Fungi morphological characteristic, *Fusarium keratoplasticum*, ITS1 and ITS4 primers, phylogenetic analysis, *Talaromyces*

Rice (*Oryza sativa* L.) is an important food crop for the population in Indonesia since they generally consume rice as staple food. Local rice such as the Kalimantan Siam 11 local rice cultivar has advantages over ordinary rice because its growth and development are suitable for local environmental conditions. However, the local rice productivity is not optimal yet because it is constrained primarily by soil fertility. The Kalimantan Siam 11 local rice cultivar is one of the local rice which is able to grow in the peatlands of South Kalimantan. One of the common problems that occurs in peatlands is the soil infertility, hence the rice production depends on continue application of chemical fertilizers which cause a decrease in soil quality (Lekatompessy and Nurjanah 2019, Dalle *et al.* 2021). Long term chemical fertilizer application can cause environmental pollution, change the physical and chemical structures of the soil, and disrupting the structure of the soil microbiome (Rahman and Dunfu 2018). Therefore, it is necessary to apply special rice cultivation techniques that are environmentally friendly and sustainable, one of which is the use of endophytic fungi.

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Endophytic fungi live in healthy plant tissue, forming colonies without causing disease symptoms (An *et al.* 2020; Wu *et al.* 2020). The endophytic fungi form mutualistic or neutral symbiosis with their host plants. The endophytic fungi obtain nutrients, ecological niches, and protection from unfavorable environmental conditions (Bamisile *et al.* 2018; Hu *et al.* 2024). Growing as a host obtains secondary metabolite compounds from the endophytic fungi, either directly or indirectly in the induction of resistance to various biotic and abiotic factors, including induction of systemic resistance (ISR). Another mechanism is through the production of bioactive phytohormone compounds such as indole-3-acetic acid (IAA), gibberellins, and cytokinin, to increase nutrient availability and the synthesis of various metabolites that function as antimicrobes, antivirus, and herbicides (Bamisile *et al.* 2018; Hu *et al.* 2024; Harrison and Griffin 2022).

Endophytic fungi are microbes that have the potential as biological fertilizers and biological control of rice plants, including local rice, hence they can be used to increase the sustainable productivity of local rice. Research on endophytic fungi in local rice has been widely carried out in Indonesia, such as local rice yellow, gondok, white sironda, and red sironda (Andriani *et al.* 2023), pulu mandoti (Syamsia *et al.* 2019), ciherang, and pandanwangi (Wiyono *et al.* 2020). However, research on endophytic fungi of the local rice cultivar Siam 11 Kalimantan has not been done. Therefore, this research aimed to study endophytic fungi diversity of the local Siam 11 rice cultivar from Kalimantan.

MATERIAL AND METHODS

Plant organ preparation. The endophytic fungi were isolated from all parts of mature plant consisting of roots, stems, leaves, brown rice, spikelets, and husks; germinated seeds which consists of radicles and celeoptiles; and seedling which consists of primary roots and shoots. Seedling and mature plant samples were obtained by growing the rice plant in Biology Department green house in Bogor, while the rest of plant organs were derived from rice plant grown in South Kalimantan peatlands. The 14 days old seedlings were harvested and primary root and shoot of the seedling were separated for fungi isolation. The mature plants were harvested at 35 days after planting. At harvest, root, stem and leaves were separated and used for fungi isolation. The germinated seeds were produced by germinating the seed in petri dishes containing sterile moist tissue and incubated for 5 days.

Endophytic fungi isolation. The endophytic fungi of local rice Siam 11 cultivar of Kalimantan isolated from the spikelets, brown rice, and husk obtained from South Kalimantan peatlands. The endophytic from another plant organ namely germinated seed include radicle and celeoptile (5 days grown in sterile zeolite), and seedling include primary root and shoot (14 days grown in sterile zeolite), root, stem and leave (30-50 days grown in polybag) were isolated from local rice Siam 11 grown in IPB University green house in Bogor. Each part was cut with a length of approximately 1 cm, while the leaf organs were cut with a size of 0.5 x 0.5 cm. All plant parts were surface sterilized using a method Sukarno *et al.* (2023) with 70% alcohol for 1 minute, 1% NaOCl for 1 minute, 70% alcohol for 1 minute, rinsed with sterile distilled water three times, and then drained on a sterile tissue. Each sterilized sample was inoculated in a PDA medium containing 0.05% chloramphenicol, then incubated for seven days and observed daily. Each growing fungus was purified by transferring it to fresh PDA media to obtain pure endophytic fungi isolates (Sukarno *et al.* 2023).

Morphological and molecular Identification. The morphology of endophytic fungi was identified by observing the colonies and morphology of the fungi macroscopically and microscopically. Colony observation was carried out after the fungus was grown on PDA media and incubated for 14 days at 28^{o}C. Observations include the characteristics of the colony such as color, growth and colony surface texture characteristics. Microscopic morphological observations were carried out using the Riddle method (1950). Each fungi isolate was grown separately on PDA placed on top of an object glass, then covered with a cover slip and incubated for 4-30 days. Characteristics of somatic and reproductive structures were observed and analyzed based on fungi

identification book (Barnett and Hunter 1972; Samson 2018; and Hocking and Pitt 2022). The isolates that could not be found in the Barnett and Hunter 1972; Samson 2018; and Hocking and Pitt, 2022 book were assisted with BLAST results from the ITS sequence, and look for references to the microscopic characters of the related genus. All morphological measurements were replicated 30 times for each isolate.

All cultures were identified molecularly using ITS rDNA sequences. DNA extraction was done using cetyl trimethyl ammonium bromide (CTAB) (Sambrook and Russell, 2001). Briefly, fungi isolates were cultured on cellophane membranes on the PDA surface at 27°C for seven days before harvesting mycelium for genomic DNA extraction. The mycelium was harvested and ground with a sterile pestle. Fungi genomic DNA was extracted using CTAB lysis buffer followed by a mixture of phenol-chloroform-isoamyl alcohol (PCI=24:1:1) and precipitated with absolute ethanol. DNA quality was measured by nanodrop and gel electrophoresis (1% agarose).

Fungi genomic DNA was used in PCR as a template to amplify the fungi ITS rDNA region using primers ITS1 (5′TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′TCCTCCGCTTATTGATATGC-3′) (White *et al.* 1990). Amplification via PCR was carried out using the method of David *et al.* (2018) and Animasaun *et al.* (2022) which was modified, namely a total volume of 40 µl containing 12 µL of sterile water, 20 µL of PCR Master Mix (my taq, Bioline), 2 µl each (10 pmol/ µl) of ITS1 and ITS4 primers, and 4 µl (250-500 ng) DNA template. The amplification reaction was carried out in 30 cycles as follows: predenaturation at 95^0C for 1.5 minutes, denaturation at 95^0C for 30 seconds, annealing at 55^0C for 30 seconds, extension at 72^0C for 1.5 minutes, final extension at 72^0C for 5 minutes, and then stored at 25^0C for 10 minutes. DNA sequencing is carried out by a sequencing service using the Sanger sequencing method. Searches and comparisons of sequence information for related endophytic fungi were conducted using the Basic Local Alignment Search Tool (BLAST) at https://blast.ncbi.nlm.nih.gov/Blast.cgi.

A phylogenetic tree construction using MEGA-X via neighbor-joining (NJ) with bootstrap 1000 (Pellegrino *et al.* 2014; Sukorini *et al.* 2021), with the out group was taken with the same family.

RESULT

Endophytic Fungi isolation. A total of 95 isolates were successfully isolated from all plant parts which were belong to 14 groups based on colony morphology characteristics. The 14 groups is further called as 14 isolates. All rice plant organs inhabited endophytic fungi except germinated seeds and seedling primary roots. There was no endophytic fungus found in germinated seeds and seedling primary roots. Three isolates of endophytic fungi were isolated from roots, three isolates from stems, six isolates from leaves, five isolates from spikelets, two isolate from brown rice, one isolated from husk, and four isolates from seedling shoots, while in the germinated seed and seedling primary root no endophytic fungi were found (Table 1 and Figure 1). The most endophytic fungi were found in the leaf organs, with six isolates, while the fewest was found in the husk with only one isolate obtained. The fourteen isolates that were successfully isolated had various colony colors, namely white, green, black, yellow, orange, and gray. The surface colony texture of fungi isolate varied, including granular, wrinkled, powdery, and velvety (Table 1 and Figure 1).

Morphological and molecular identification. Fourteen isolates were identified, 11 of which formed asexual reproductive structures with spores, while three isolates only had sterile mycelium after routine observation for one month therefore the three isolates cannot be identified morphologically. The 11 isolates which formed asexual reproductive structures could be identified into six genera namely *Cladosporium, Penicillium, Talaromyces, Fusarium, Sarocladium* and *Simplicillium.* Isolates 0A and 1G were identified as *Cladosporium*, isolates A1.1 and A2.1 were *Fusarium*, isolates 6A and 4D were *Penicillium*, and isolates 7D, 7G, and 9D were *Talaromyces,* isolates 2G were *Sarocladium* and isolates 2D were *Simplicillium* (Table 1). *Cladosporium* is the genus inhabit various plant organs, inhabit almost all plant parts such as

leaves, roots, stems, husks, and seedling shoot. Meanwhile, the genus with a low distribution was *Fusarium*, only inhabits seedling shoot. A total of 3 isolates, namely isolate 1A from roots, isolate 8B from stems, leaves, spikelets, brown rice, and husks, isolate 0G from spikelets, and brown rice, from microscopic structure only sterile mycelia therefore could not be identified morphologically after 30 days observation.

Cladosporium sp.1 0A has a pale green upper surface of the colony and a black lower surface, and the colony has a soft texture and is irregular. The hyphae septate with a diameter of 2.7-5.69 µm. Conidiophores are long, brown, upright, branching variously near the ends, and clustered with 34.17-106.23 µm lengths. Conidia are brown, ovoid with diameter ranging 3.49-3.63 x 3.99-8.81 µm.

Cladosporium sp. 2 1G has a dark green upper surface of the colony, which gradually turns black and has a rough texture and is irregular. The lower surface of the colony is solid black. The hyphae are septate with a hyphal diameter of 3.12-5.57 µm and the distance between septa is 9.63-21.58 µm, conidiophores long, dark, upright, diverse branches near the ends, grouped or single having a length of 20.67-56.93 µm, conidia dark, spherical with a diameter of 3.09 x 3.79 – 4.87 x 5.65 µm.

Penicillium sp. 1 6A has a pale yellow upper surface of the colony and a soft texture. The lower surface of the colony is brownish-yellow. Irregular colony shape. The hyphae are septate with a wide diameter of 2.5-4.86 µm. It has a long, single, monoverticillate conidiophore length of 20.22-93.42 µm. The phialides are flask shaped, gradually tapering to neck (7.55- 12,32 µm). Chain conidia are round, size of 2.97 x 2.97 – 4.14 x 4.51 µm in diameter.

Penicillium sp. 2 4D has a soft texture with a dark green upper surface of the colony, while the lower surface is yellow. The hyphae septate has a diameter of 2.4-5.27 µm. The conidiophores (12.04-48.54 µm) are shorter than *Penicillium* sp. 1 6A. The phialides are flask shaped, gradually tapering to neck (6.02-12.76 µm). Conidia was round 2.5 x 3.06 – 3.15 x 3.37 µm and in chain.

Tabel 1. The occurrence of endophytic fungi isolates in Kalimantan Siam 11 local rice cultivar

Fungi name	Plant organ									
							Germinated seed		Seedling	
	Root	Steam	Leaf	Spikelets	Brown rice	Husk	Radicle	Celeoptile	Primary Root	Shoot
Cladosporium sp.1 0A	+	+	+	-	-	-	-	-	-	-
Cladosporium sp.2 1G	-	-	-	+	-	-	-	-	-	+
Fusarium sp.1 A1.1	-	-	-	-	-	-	-	-	-	+
Fusarium sp.2 A2.1	-	-	-	-	-	-	-	-	-	+
Isolated 0G	-	-	-	+	+	-	-	-	-	-
Penicillium sp.1 6A	+	-	-	-	-	-	-	-	-	-
Penicillium sp.2 4D	-	-	+	-	-	+	-	-	-	-
Isolated 1A	+	-	-	-	-	-	-	-	-	-
Isolated 8B	-	+	+	+	+	-	-	-	-	-
Sarocladium sp. 2G	-	+	-	+	-	-	-	-	-	-
Simplicillium sp. 2D	-	-	+	-	-	-	-	-	-	+
Talaromyces sp.1 7D	-	-	+	-	-	-	-	-	-	-
Talaromyces sp.2 7G	-	-	-	+	-	-	-	-	-	-
Talaromyces sp.3 9D	-	-	+	-	-	-	-	-	-	-
Total	**3**	**3**	**6**	**5**	**2**	**1**	**0**	**0**	**0**	**4**

Note: + = Present, - = absent

Table 2. BLAST analysis of endophytic fungi Kalimantan Siam 11 local rice cultivar

Isolate	BLAST result	Query (%)	Similarity Index (%)	BLAST result Accession number
Cladosporium sp.1 0A	*C. oxysporum*	100	99. 78	NR_152267.1
Cladosporium sp.2 1G	*C. coloradense* CPC 22238	100	98.67	RS 156347.1
Fusarium sp.1 A1.1	*F. keratoplasticum* FRC S-2477	100	100.00	NR_130690.1
Isolate 0G	*F. keratoplasticum* FRC S-2477	100	100.00	NR_130690.1
Fusarium sp.3 A2.1	*F. humuli* CGMCC 3. 1974	100	99.76	NR_164598.1
Penicillium sp.1 6A	*P. beferidium* NRRL 710	100	99.79	NR_138263.1
Penicillium sp.2 4D	*P. citrinum* CBS 2323.38	100	100.00	NR_121224
Isolate 1A	*Poaceascoma lochia* BRIP 71546	81	92.67	NR_173241.1
Isolate 8B	*Pseudopestalotiopsis simitheae* MFLUCC 120121	93	99.78	NR_11716.1
Sarocladium sp. 2G	*Sarocladium oryzae* CBS 180.74	100	100.00	NR_145045.1
Simplicillium sp. 2D	*Simplicillium obclavatum* CBS 133.74	100	98.83	NR_111099.1
Talaromyces sp.1 7D	*T. macrosporus* CBS 317.63	100	98.85	NR_145155.1
Talaromyces sp.2 7G	*T. angelicus* NRRL 2750	100	100.00	MT906348.1
Talaromyces sp.3 9D	*T. pinophilus* CBS 631.66	100	99.46	NR_111691.1

Talaromyces sp. 2 7D has an orange upper surface of the colonies, soft textured colonies. The underside of the colony is red and irregular. It has septate hyphae with a wide diameter of 1.76-3.41 µm. The conidiophores are 12.47-24.09 µm. The phialides are parallel side, abruptly tapering to neck (6.35- 14.32 µm). The chain conidia are round with a 1.67 x 2.75 – 3.28 x 4.38 µm diameter.

Talaromyces sp. 2 7G has a soft texture with a yellow colony surface. The underside of the colony is white. The colony shape is round. It has septate hyphae with a wide diameter of 1.5-5.93 µm. The conidiophores are longer than *Talaromyces* sp. 2 7D (21.76-43.93µm). The phialides are parallel side, abruptly tapering to neck (10.79-12 µm). The chain conidia are round with a 2.25 x 3.42 – 3.3 x 5.96 µm diameter.

Talaromyces sp. 3. 9D has a green colony surface and white on the edges with a soft texture. The underside of the colony is white. The colony shape is round. It has septate hyphae with a wide diameter of 1.65-3.88 µm. The conidiophores (10.2-15.45 µm) are shorter than *Talaromyces* sp. 1 7D and *Talaromyces* sp. 2 7G. The phialides are parallel side, abruptly tapering to neck (4.44-4.69 µm). The conidia are oval with a 1.91x1.94–3.44x3.74 µm.

Fusarium sp.1 A1.1 has a soft colony texture, white, cottony, and irregular on the upper surface, while the lower surface is brownish yellow. The septate hyphae have a diameter of 9.46- 11.52 µm. Simple conidiophores were hyaline, slender, 27.68-97.16 µm length. The length of microconidia was 8.65-9.27 µm, and macroconidia was fusiform, 7.55-11.88 µm x 9.46-11.52 µm.

Fusarium sp.2 A2.1 has a soft texture and irregular, white bone colonies on the upper surface. The lower surface was brownish-yellow. The septate hyphae have a diameter of 2.44-3.76 µm. Simple conidiophores were hyaline, slender, and had a 27.68-97.16 µm length. Microconidia was 7.55-8.65 µm x 15.07-16.87 µm. Macroconidia were slightly curved, hyaline, 3.21-3.80 µm x 20.94-30.38 µm.

Siplicillium sp. 2D has irregular, white, cottony colony and a soft texture on the upper surface. The lower surface of the colony is brownish-yellow. The septate hyphae have a diameter of 1.13-2.55 µm. Hyaline, slender, and simple conidiophores have 27.68-97.16 µm length. The conidia are formed sympodial, with a diameter of 1.11- 2.24 in length and 1.13-2.55 in

width.

Sarocladium sp. 2G has a soft texture and irregular, white cottony colonies on the upper surface; on the lower surface, it is brownish-yellow. The septate hyphae have a diameter of 1.14-2.57 µm. Simple conidiophores were hyaline and slender and 27.68-97.16 µm long. The oval-shaped conidia have 3.27-6.51 µm x 1.14-3.03 µm.

Isolated 1A has a brownish-gray upper surface of the colony and has a soft texture. The lower surface of the colony is grayish-black. The colony shape is round. It has septate hyphae with a 2.9 -4.6 µm diameter. Conidia are absent after observations for one month.

Isolated 8D has white, cottony colonies on the upper surface. It has a soft texture. The septate hyphae have a diameter of 3.9-5.49 µm. Conidiophores and conidia are absent after observations for one month.

Isolated 0G has a gray upper surface of the colony and has a soft texture. The lower surface of the colony is grayish-black. It has septate hyphae with a 3.07 - 5,23 µm diameter. Conidia was absent after observations for one month.

Molecular identification was done using a BLAST and phylogenetic tree analysis using DNA sequences of ITS region of rDNA. All of the endophytic isolates are identic to a database of Gen-Bank species, which similarity varies from 98-100%, 81-100% for query coverage (QC), and an E-value of 0.0 - (5e-114). The E-value of 11 isolates was 0.0, and the E-value of the other two isolates were 6e-117 and 5e-114 (Table 2).

The results showed that the isolated endophytic fungi belong to 8 genera and 13 species. The fungi genera were *Cladosporium, Paeoscoma, Penicillium, Talaromyces, Simplicillium, Fusarium, Pseudopestalotiopsis*, and *Sarocladium*. Three isolates that could not be identified morphologically were identified molecularly as belonging to the genus *Fusarium, Poaceascoma,* and *Pseudopestalotiopsis.*

Further molecular analysis using phylogenetic tree showed similar results with that of BLAST (Figure 2-9). Fungi isolates have a bootstrap value of more than 75%, indicated very similar.

The fungi were identifies as C*ladosporium oxysporum* 0G, *C. coloradense* 1G, *Fusarium keratoplasticum* A1.1 and 0G , *F.humuli* A 2.1, *Penicillium brefeldianum* 6A, *P.citrinum* 4D, *Poaceascoma lochia* 1A, *Pseudopestaotiopsis simitheae* 8B, *Sarocladium oryzae* 2G, *Simplicillium obclavacum* 2D, *Talaromyce macrosporu* 7D and *T. pinophilus* 9D. The isolate which had bootstrap value less than 75% was identifies as *Talaromyces angelicus* 7G (69%). The two isolates that had low E-value in BLAST analysis were identified as *P. citrinum* 4D and *Poaceascoma lochia* 1A using phylogenetic analysis (Figure 2- 9).

DISCUSSION

Endophytic fungi inhabit almost all organs of Kalimantan Siam 11 local rice cultivar. The most fungi are found in the leaves and the least in the husk. Various factors can influence the presence of endophytic fungi, including variations in plant cultivars, sampling locations, rainfall, and cultivation system (David *et al.* 2016). Su-Han *et al.* (2019) reported that different nutritional content, anatomy and duration of exposure to endophytic fungi airborne spores of each host plant tissue can influence differences in their endophytic fungi communities.

Based on the morphological identification of 14 fungi group isolates, 11 isolates can be identified by morphological characteristics and identified into six genera namely *Cladosporium, Penicillium, Talaromyces, Simplicillium, Sarocladium* and *Fusarium*). Three isolates did not form reproductive structures, only sterile mycelia, therefore they could not be identified. Sterile mycelia are a group of true endophytic fungi that do not form asexual reproductive structures (Reis *et al.* 2022). *Cladosporium* was found in all parts of the rice samples used as a source of isolation, except in the brown rice, husks and germinated seeds. In contrast, *Fusarium* is only obtained in seedling shoot.

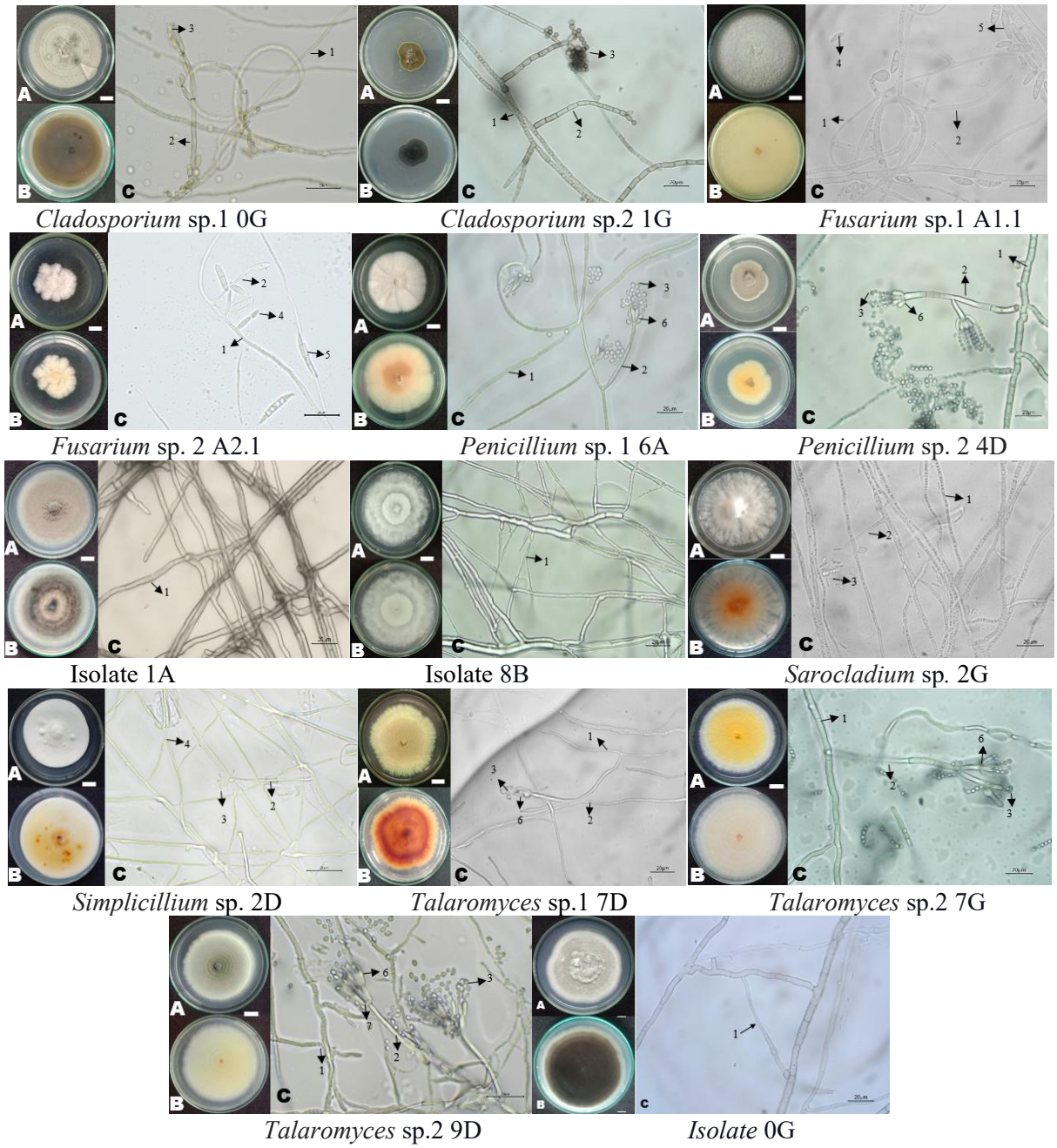

Fig 1 Colony and microscopic structures of endophyte fungi of Kalimantan Siam 11 local rice cultivar grown on PDA at 14 days after inoculation at 28°C. The top surface of the colony (A), the bottom surface of the colony (B), microscopic structures (C), hyphae (1), conidiophores (2) conidia (3), microconidium (4), macroconidium (5), phialide (6), and metula (7). Scale line = 1 cm (A-B), 20 µm (C).

Other fungi, such as *Penicillium, Simplicillium, Sarocladium* and *Talaromyces* were found in the same or different parts of the host plant samples. The presence of endophytic fungi is random and can be found in all organs of the rice plant (Trizelia *et al.* 2023; Wijesooriya and Deshappriya 2016). The *Penicillium* and *Fusarium* were reported as endophytic fungi of local rice varieties of Kuruluthuda (Wijesooriya and Deshappriya 2016) and rice cultivars of Khao Jow Hawm Suphan Buri and Pathum Thani 80 (Leewijit *et al.* 2016). Furthermore Potshangbam *et al.* (2017) reported that *Sarocladium, Talaromyces*, and *Cladosporium* are endophytic fungi in rice plants. *Sarocladium oryzae*

obtained in this study were reported as endophytic fungi in Ciherang rice cultivar by Sunariasih *et al.* (2014), who also reported *Penicillium citrinum* and *Cladosporium* sp. However, *Fusarium* was not found in the Ciherang rice cultivar. Literature studies have not yielded any reports regarding *Simplicillium* as the endophytic fungi of rice and other plants.

Molecular identification of the 14 isolate was supported by similarity data from BLAS and bootstrap values from phylogenetic trees. The BLAST analysis showed that all isolates had 98-100% similarity. Phylogenetic tree analysis showed that 12 isolates were related with bootstrap values between 77 -100% (*Cladosporium oxysporum* 0G, *C. coloradense* 1G, *Fusarium keratoplasticum* strain 1 A1.1 and strain 2 0G, *F.humuli* A 2.1, *Penicillium brefeldianum* 6A, *P. citrinum* 4D, *Poaceascoma lochia* 1A, *Pseudopestaotiopsis simitheae* 8B, *Sarocladium oryzae* 2G, *Simplicillium obclavacum* 2D, *Talaromyce macrosporus* 7D and *T. pinophilus* 9D), meanwhile *Talaromyces angelicus* 7G the bootstrap values was 72%. The BLAST analysis similarity value of more than 96% indicates high similarity (Lam *et al.* 2019). Bootstrap values of 75-95% indicate high similarity (Lombard *et al.* 2019; Sabahi *et al.* 2023).

Five species obtained in this study have not been reported as endophytic fungi in rice and other plants, namely *Cladosporium coloradense* 1G, *Poaceascoma lochii* 1A, *Talaromyces macrosporus* 7D, *Pseudopestaotiopsis simitheae* 8B and *Simplicillium obclavacum* 2D. Some of these fungi have been reported, as a result of isolation from animal and human pathogens, (Bensch *at al.* 2018; Yamashita *et al.* 2019). Seven species have not been reported as endophytic fungi from rice but they have been reported as endophytic fungi of other plants, the fungi were *Cladosporium oxysporum* OA reported as endophytic fungi on *Aglaia odorata* (Sugijanto and Dorra 2016), *Penicillium brefeldianum* 6A reported as endophytic fungi on *Syzygium zeylanicum* (Syarifah *et al.* 2021). *Penicillium citrinum* 4D was reported as an endophytic fungus on *Azadirachta indica* (Kumaria *et al.* 2021). *Talaromyces pinophilus* 9D was reported as endophytic fungus on *Zingiberaceae* (Amany *et al.* 2021). *Fusarium humuli* A2.1 was reported as an endophytic fungus on *Vitex rotundifolia L.* (Park *et al.* 2023). *Fusarium keratoplasticum* A1.1 and 0G was reported as an endophytic fungus on *Dendrobium* (Surendra *et al.* 2020). *Talaromyces angelicus* 7G was reported as an endophytic fungus on dried roots of *Angelica gigas* and *Cnidium officinale* (Sang *et al.* 2013).

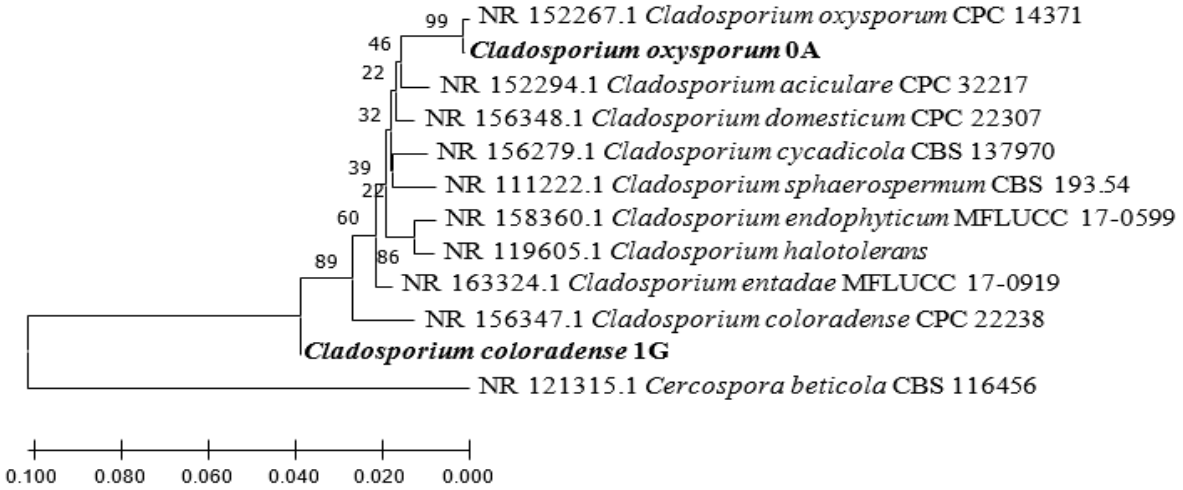

Fig 2 Phylogenetic tree of *Cladosporium oxysporum* 0A and *Cladosporium coloradense* 1G. *Cercospora beticola* CBS 116456 as an outgroup.

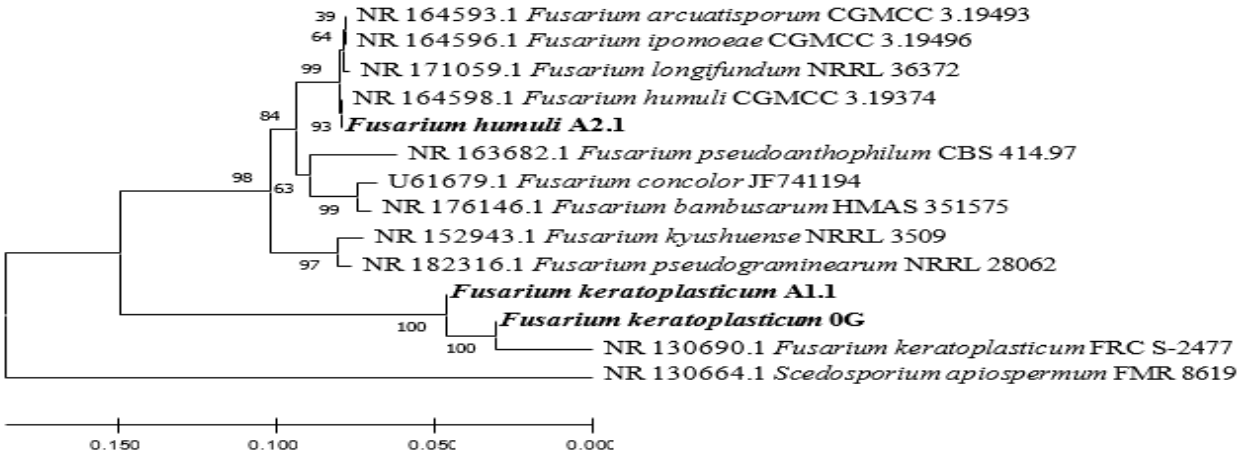

Fig 3 Phylogenetic tree *Fusarium humuli* A2.1 and *Fusarium keratoplasticum* A1.1 and 0G and *Scedosporium apiospermun* FMR 8619 as an outgroup.

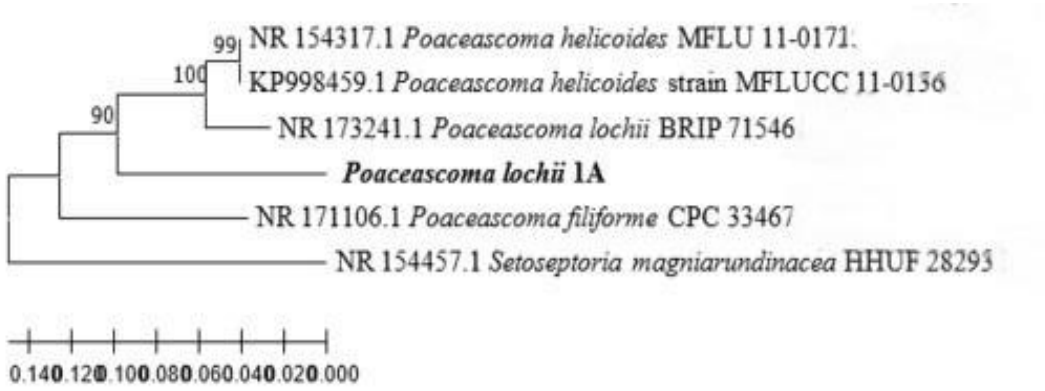

Fig 4 Phylogenetic tree of *Poaceascoma lochii* 1A and *Setoseptoria magniarundinacea* HHUF 28293 as an outgroup.

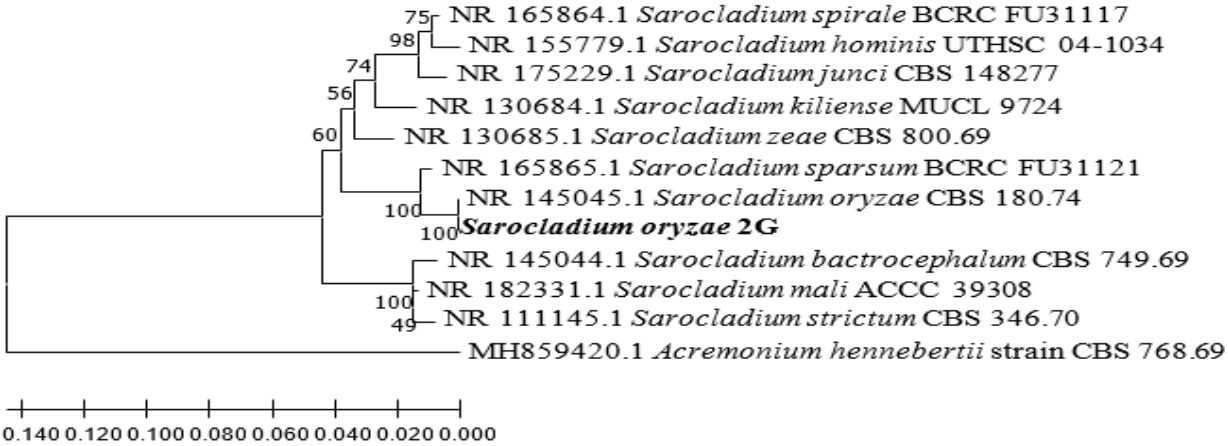

Fig 5 Phylogenetic tree *Sarocladium oryzae* 2. *Acremonium hennebertii* CBS 768.69 as an outgroup

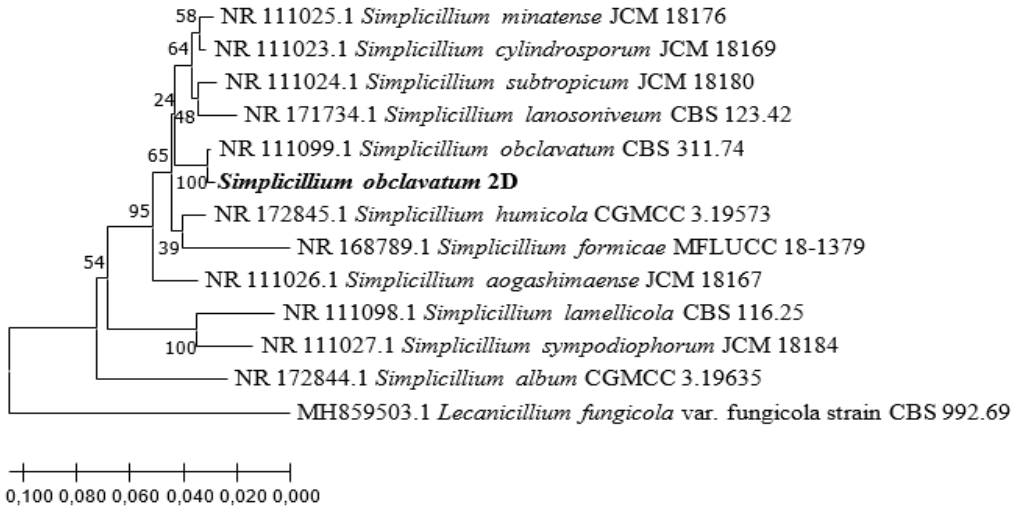

Fig 6 Phylogenetic tree *Simplicillium obclavacum* 2D. *Lecanicillium fungicola* CBS 992.69 as an outgroup.

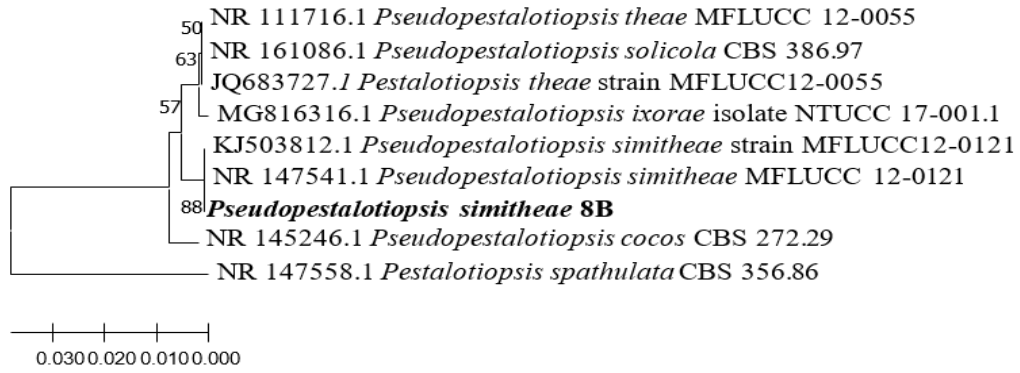

Fig 7 Phylogenetic tree *Pseudopestalotiopsis simitheae* 8B. *Pestalotiopsis spothulata* CBS 356.86 as an outgroup.

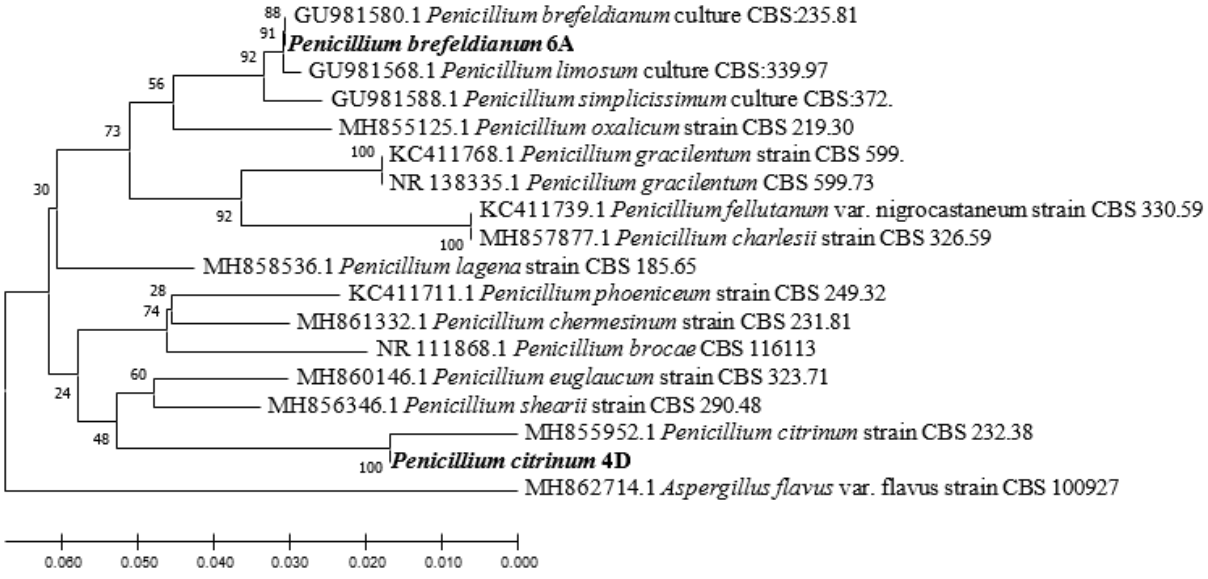

Fig 8 Phylogenetic tree *Penicillium brefeldianum* 6A and *Penicillium citrinum* 4D. *Aspergilus flavus* CBS 100927 as an outgroup.

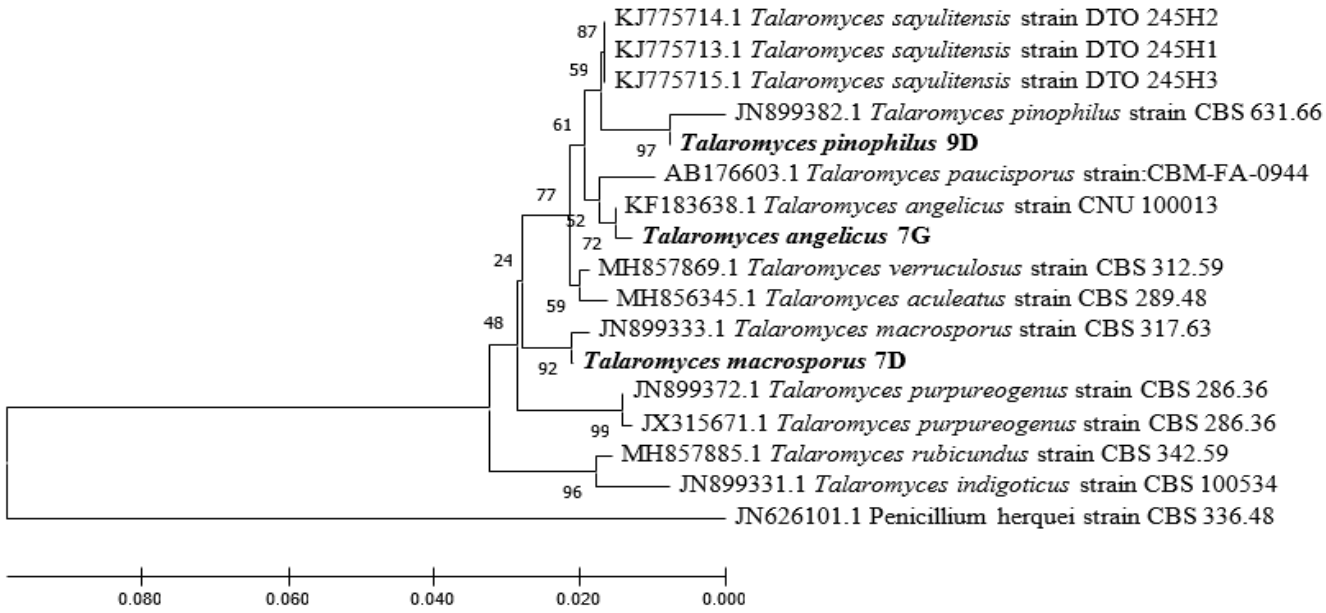

Figure 9. Phylogenetic tree *Talaromyces pinophilus* 9D*, Talaromyces angelicus* 7G*, Talaromyces macrosporus* 7D*. Penicilium herquei* CBS 336.45 as an outgroup.

CONCLUSION

Fourteen isolates of the fungus were successfully isolated from the Kalimantan Siam 11 local rice cultivar and identified based on combined morphological and molecular characteristics into thirteen species. The fungi were C. *oxysporum* 0G, *C. coloradense* 1G, *F. keratoplasticum* A1.1 and 0G, *F.humuli* A 2.1, *P.brefeldianum* 6A, *P.citrinum* 4D, *Poaceascoma lochia* 1A, *Pseudopestaotiopsis simitheae* 8B, *Sarocladium oryzae* 2G, *Simplicillium obclavacum* 2D, *Talaromyce macrosporu* 7D and *T. pinophilus* 9D and *Talaromyces angelicus* 7G. Almost all rice plant organs were inhabited by endophytic fungi. The most isolates were found in the leaves which occupied by 6 isolates and the fewest isolates were found in the husk with only 1 isolate. The *Cladosporium* is found in almost all parts of the plant except the brown rice and husk. The *Cladosporium* is consisting of two species, namely *Cladosporium oxysporum*, which is often found in the roots, leaf stems and seedling, and *Cladosporium coloradense,* which is found in the spikelets and seedling pimary roots. Meanwhile, the fungi genus with the lowest host range is the *Poaceascoma lochia* which is only found in the roots.

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