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Original Article

Molecular Identification of Mycorrhizal Fungi Species in the Roots of *Azadirachta excelsa* Trees in South Bengkulu Regency, Bengkulu Province

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Abstract

Azadirachta excelsa trees are cultivated in many regions of South Bengkulu Regency. This species is known to form associations with mycorrhizal fungi, which assist the trees in absorbing nutrients and water while protecting their roots from pathogenic attacks. The present study aimed to identify the types of mycorrhizal fungi associated with the roots of *A. excelsa* trees through biomolecular analysis in three villages of South Bengkulu Regency, Bengkulu Province. Root samples were collected from *A. excelsa* stands located in Pagar Dewa Village, Manna Subdistrict; Tanjung Eran Village, Pino Subdistrict; and Batu Ampar Village, Kedurang Subdistrict, South Bengkulu Regency. Root DNA extraction was conducted using a Geneaid Fungal Kit, followed by Polymerase Chain Reaction (PCR) with forward ITS5.8S and reverse ITS4 primers. Electrophoresis was performed using a 1.5% agarose gel in TAE 1X buffer at 100V for 32 minutes, and sequencing was carried out at 1st BASE PT Genetic Science Indonesia. The resulting sequences were then compared with those available in the GenBank database, and a phylogenetic tree was reconstructed using MEGA XI. Sequencing results revealed three species of mycorrhizal fungi associated with *A. excelsa* roots from samples collected in Pagar Dewa. These included a species from the Agaricaceae family (*Delicatula integrella*), belonging to the order Agaricales, class Agaricomycetes, and division Basidiomycota. Two additional species—*Basidiomycota* sp. (MT645155.1), *Uncultured fungus* (MF568791.1), *Uncultured fungus* (MT672409.1), and *Uncultured fungus* (MH636718.1)—were classified as unclassified Basidiomycota or could not be definitively categorised in the GenBank database.

Keywords: *Azadirachta excelsa; Basidiomycota; Biomolecular; Mycorrhiza; Sequencing*

Introduction

Azadirachta excelsa is a prominent forestry species cultivated across several regions of Bengkulu Province. The tree holds significant cultural value for the Bengkulu community, particularly among the Lembak and Rejang tribes. The dwellings of these tribes are traditionally constructed using timber, with *A. excelsa* being a preferred material. According to Siahaan *et al*. (2011), *A. excelsa* is a fast-growing tree with a growth cycle of 10–15 years and is regarded as a source of high-quality carpentry wood. Additionally, this species produces the chemical compound azadirachtin, particularly concentrated in its seeds (Kanokmedhakul *et al*., 2005). This compound serves as a base ingredient in pharmaceuticals and natural pesticides that are safe for humans, the environment, and beneficial insect species (Lim & Bottrell, 1994). It is also highly effective in controlling approximately 200 types of insects (Premono & Lestari, 2013).

A. excelsa thrives in Bengkulu Province, typically growing on nutrient-poor ultisol soils. The presence of mycorrhiza in plant roots greatly enhances nutrient uptake, particularly phosphorus, in such soils.

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The composition of the soil and surrounding vegetation influences the type of mycorrhiza present, resulting in a site-specific symbiotic relationship. Therefore, identifying the appropriate type and species of mycorrhiza is essential for promoting optimal plant growth. Some *A. excelsa* trees are known to associate naturally with mycorrhizal fungi. Previous studies have shown that this tree species forms relationships with numerous mycorrhizal taxa. Huat *et al*. (2002) reported that *A. excelsa* exhibited an 81.25% association rate with mycorrhizal fungi. Similarly, Chubo (2009) observed the presence of mycorrhizal associations in *A. excelsa* seedlings. However, despite these findings, the relationship between *A. excelsa* and mycorrhizal fungi remains poorly understood. A molecular investigation by Anwar and Ketaren (2023) identified ten mycorrhizal species associated with *A. excelsa* roots: *Psathyrella* sp., *Mycena* sp., *Filoboletus manipularis*, *Mycena amicta*, *Tricholomataceae* sp., *Agaricales* sp., *Mycena pura*, *Mycena rosea*, *Mycena citrinomarginata*, and *Favolaschia manipularis*.

Mycorrhizal fungi play a vital role as plant mutualists, significantly influencing forest community composition worldwide (Delavaux *et al*., 2017; Steidinger *et al*., 2019; Delavaux *et al*., 2023). These fungi have maintained mutualistic relationships with plants for at least 450 million years, providing essential nutrients in exchange for carbon derived from the host plant's photosynthesis (Redecker *et al*., 2000). Mycorrhizal fungi also play a crucial role in regulating plant nutrition and carbon cycling (Phillips *et al*., 2013; Anwar *et al*., 2020). Their hyphae extend far beyond the reach of plant roots, enabling them to access distant sources of nutrients and water and transfer these resources to the host plant (Peng, 2013). Most forests on Earth are dominated by tree species that form either Arbuscular Mycorrhiza (AM) or Ectomycorrhiza (EM) associations (van der Heijden *et al*., 2015; Parwito & Anwar, 2024).

Molecular methods are now widely employed to identify different types of mycorrhizal fungi and to gain a deeper understanding of their diversity and ecological functions (Tedersoo *et al*., 2016). These techniques can also be combined with morphological studies of fruiting bodies to assist in identifying specific genera or species of ectomycorrhizal fungi by analysing their DNA (Riviere *et al*., 2007). The identification of ectomycorrhizal fungi can be achieved by comparing their DNA sequences with known internal transcribed spacer (ITS) or mitochondrial ribosomal sequence databases (Kõljalg *et al*., 2005). Furthermore, molecular techniques can be applied to ectomycorrhizal fungi samples in the form of mycelium, as many fungi colonise primarily as mycelia rather than producing fruiting bodies. Some ectomycorrhizal fungi commonly present in soil rarely produce fruiting bodies, while others that typically form fruiting structures may not be found in soil environments (Dahlberg, 2001). This study was conducted to identify and characterise the species of mycorrhizal fungi molecularly associated with the root systems of *A. excelsa* trees across three villages in South Bengkulu Regency, Bengkulu Province.

Materials and Methods

Sampling locations were selected at three sites based on the presence of more than 30 *A. excelsa* trees. The first location was Pagar Dewa Village, Kota Manna Subdistrict, South Bengkulu Regency. This area was situated along the roadside and was easily accessible by car. The plot featured an agroforestry planting scheme comprising *A. excelsa* trees intercropped with salak (snake fruit) plants. The second location was Tanjung Eran Village, Pino Subdistrict, South Bengkulu Regency. Due to limited road access, this area could be reached partly by car and then on foot for approximately 200 metres. This site also featured an agroforestry system combining *A. excelsa* trees with oil palm (*Elaeis guineensis*) plants. The third location was Batu Ampar Village, Kedurang Subdistrict, South Bengkulu Regency. This site was located along the roadside and was accessible by vehicle. The agroforestry planting scheme at this site also integrated A. excelsa trees with oil palm plants. Figure 1 and table 1 presents a map illustrating the sampling locations and table 1 summarizes characteristics of the sampling trees.

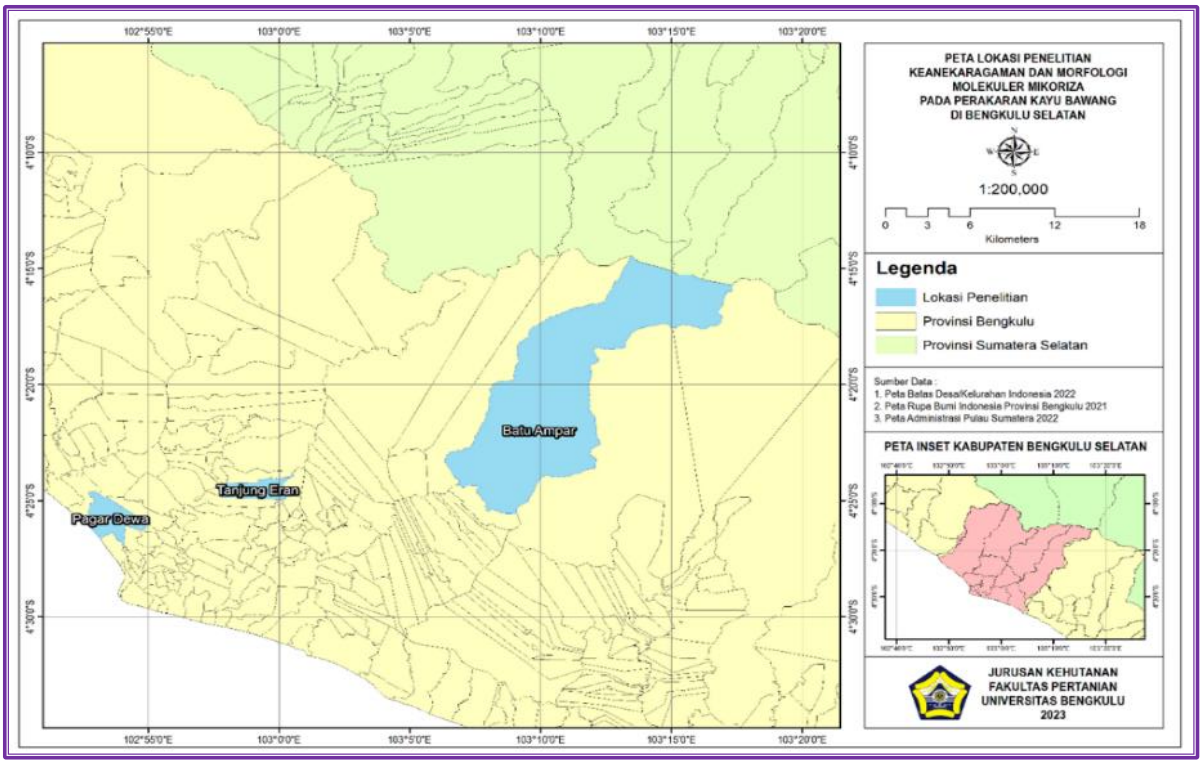

Figure 1: *Sampling Sites*

Table 1: *Characteristics of the Sampling Trees*

#	Location	Sampling Code	Coordinates	Height (m)	Diameter (cm)	Neighbor Plants
1	Pagar Dewa Village	DPD1	"S04°26'06.71" E102°53'55.22"	14	16.88	Snake Fruit
2		DPD2	"S04°26'06.71" E102°53'55.22"	22	25.16	
3		DPD6	"S04°26'06.58" E102°53'54.69"	25	16.88	
4		DPD8	"S 04°26'06.53" E 102°53'54.56"	29	20.38	
5		DPD10	"S04°26'06.44" E 102°53'55.13"	25	19.43	
6		DTE2	"S04°25'03.12" E102°59'31.67"	20	31.85	Oil Palm Trees
7		DTE3	"S04°25'02.87" E102°59'31.33"	21	26.43	
8	Tanjung Eran Village	DTE6	"S04°25'02.06" E102°59'30.00"	15	29.94	Oil Palm Trees
9		DTE8	"S04°25'02.72" E102°59'32.26"	15	25.48	
10		DBA1	S04°23'39.30" E103°08'17.00"	40	54.14	
11		DBA2	"S04°23'39.71" E103°08'16.76"	41	38.85	
12		DBA3	"S04°23'39.74" E103°08'16.99"	40	52.55	
13	Batu Ampar Village	DBA4	"S04°23'40.13" E103°08'17.10"	42	48.41	Oil Palm Trees
14		DBA5	"S04°23'40.75" E103°08'17.19"	40	47.77	
15		DBA8	"S04°23'40.44" E103°08'17.56"	50	56.69	

Five grams of root samples were collected from *A. excelsa* trees—five from Pagar Dewa Village, four from Tanjung Eran Village, and six from Batu Ampar Village. Root sampling was conducted by excavating soil in the root zone, approximately 50–100 cm from the main trunk of each sample tree. Roots were collected from four directions corresponding to the cardinal points of the wind at a depth of 20–30 cm. The roots obtained were fresh root hairs exhibiting bright coloration and measuring approximately 10 cm in length. Each root sample was placed in a labelled plastic bag for subsequent laboratory processing. The samples were then stored in a freezer at temperatures ranging from −20°C to −30°C until DNA extraction was carried out.

Soil samples were collected from the same locations where root samples had previously been obtained. The soil surface was first cleared, and samples were taken using a shovel. A total of 1 kg of soil was collected at a depth of 20 cm from the rhizosphere of ten trees. Non-soil components were removed from the samples, which were then placed in labelled plastic bags for further analysis.

The soil samples collected from the research sites were placed in labelled plastic bags and air-dried at room temperature (20–25°C). These samples were used to analyse soil texture, pH, and nutrient content, including organic carbon (C), nitrogen (N), phosphorus (P), and potassium (K). The analyses were conducted at the Soil Laboratory of the Bengkulu Agricultural Instrument Standards Application Centre (BPSIP). The aim of this analysis was to determine the availability of nutrients and assess their influence on the symbiotic relationship between mycorrhizal fungi and *A. excelsa* trees.

Field-collected root samples were initially stored in a freezer and subsequently dried at room temperature to allow temperature equilibration. The roots were then cleaned with tap water to remove any adhering soil or debris. Brightly coloured, soft-textured young roots (fine roots) were selected, cut into 2 cm segments, and weighed to 0.03 grams for molecular analysis. Fifteen root samples underwent identical preparation procedures. For molecular biology processing, DNA was extracted from root samples by grinding them in liquid nitrogen until a fine powder was obtained. The samples were then processed using the Geneaid Fungal Kit protocol. The powdered root samples were transferred into bead-beating tubes labelled with their corresponding codes. Subsequently, 600 µL of GT Buffer was added, and the mixture was vortexed for 1 minute. Five µL of RNase A (50 mg/mL) was then added to each tube, followed by incubation at 70°C for 10 minutes, with the tubes inverted every 3 minutes. Afterwards, 100 µL of PR Buffer was added, and the tubes were briefly vortexed again. Following the detailed instructions in the Geneaid Fungal Kit protocol, each step was meticulously carried out until a DNA extraction solution suitable for amplification was obtained (Geneaid Fungal Kit Manual).

DNA samples at the internal transcribed spacer (ITS) locus were amplified using a Polymerase Chain Reaction (PCR) machine with forward ITS 5.8S primers (5'-CAAGCAGAAGACGG CATACGAGAT-NNNNNNNNNNNN-AGTCAGTCAG -GG AACTTTYRRCAAYGGATCWCT-3') and reverse ITS4 primers (5'-AATGATACGGCG ACCACCGAGATCTACAC-TATGGTAATT-AA-AGCCTCCGCTTA TTGATATGCTTAART-3') (Taylor *et al*., 2016). The reaction mixture consisted of 25 µL of GoTaq Green Master Mix (Promega, USA), 5 µL of DNA template from the extraction, 2 µL each of forward and reverse primers, and nuclease-free H_2O to reach a final volume of 50 µL. PCR conditions included an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 30 seconds at 95°C, 45 seconds at 58°C, and 1 minute at 72°C. A final elongation step was performed at 72°C for 10 minutes, followed by a hold at 4°C. The amplified DNA was visualised by electrophoresis on a 1.5% agarose gel in 1X TAE (Tris-Acetate-EDTA) buffer at 100 V for 32 minutes. The final sequencing was conducted at 1st BASE PT. Genetika Science Indonesia.

The nucleotide sequences (forward and reverse) obtained from sequencing were edited and aligned using the ClustalW algorithm in the MEGA 11 software program. The resulting DNA sequences were compared against the GenBank database of the National Center for Biotechnology Information (NCBI) through the Barcode of Life Data System (BOLD System) to determine species similarity or identity. A phylogenetic tree was reconstructed using MEGA 11 software employing the neighbour-joining method with 1,000 bootstrap replications.

Results and Discussion

The chemical and physical analysis of the soil revealed no significant differences between the macro elements of soil samples from the three sampling locations. Table 2 shows the soil analysis results.

Table 2: *Chemical Soil Analysis Results*

Number	Soil measurement		Sampling Location		
			Pagar Dewa	**Tanjung Eran**	**Batu Ampar**
1	Soil pH	H_2O	5.09 (3.9-5.9)	5.00 (3.9-5.9)	5.48 (3.9-5.9)
			Optimum	Optimum	Optimum
		KCl	4.12 (3.9-5.9)	3.84 (3.9-5.9)	4.55 (3.9-5.9)
			Optimum	Optimum	Optimum
2	C (%)		2.03 (2.02-3.00)	3.07 (3.01-5.00)	5.90 (3.01-5.00)
			Medium	High	High
3	N (%)		0.21 (0.21-0.50)	0.30 (0.21-0.50)	0.49 (0.21-0.50)
			Medium	Medium	Medium
4	P (ppm)		2.27 (<10)	0.95 (<10)	0.64 (<10)
			Low	Low	Low
5	K (mg/100gram)		49.49 (41-60)	30.17 (21-40)	32.65 (21-40)
			High	Medium	Medium

This study utilised the ITS (Internal Transcribed Spacer) 5.8S gene sequence. The Internal Transcribed Spacer is one of the molecular DNA markers commonly employed to analyse phylogenetic relationships in both higher and lower plants. The ITS region from the nuclear genome is widely used in taxonomy and molecular phylogenetics because it is easy to amplify and exhibits high levels of variation, even among closely related species (Kitur *et al*., 2025). Eukaryotic organisms possess two ITS regions: ITS-1, located between the 18S and 5.8S genes, and ITS-2, situated between the 5.8S and 28S genes. These ITS regions frequently undergo mutations or sequence changes, leading to differences or variations among species. However, the use of a single marker gene for species identification does not always guarantee accurate taxonomic resolution. In certain genera, identification cannot be achieved using only one type of marker gene. Some taxa therefore require the combination of two or more molecular markers to ensure precise identification, as each family, genus, and species possesses unique genetic characteristics.

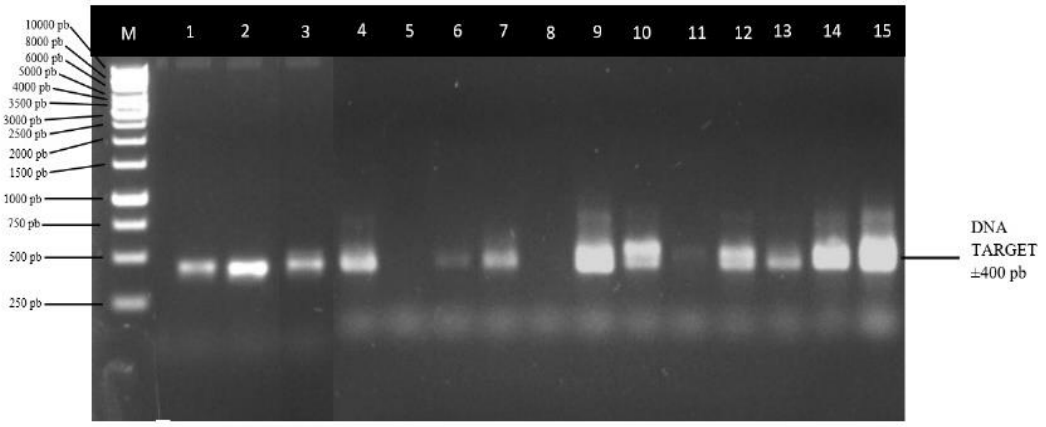

Figure 2*: DNA Electrophoresis Results of ITS Genes in 15 Root Samples*
(left to right: DTE2, DPD2, DBA8, DBA3, DBA1, DBA2, DPD8, DPD1, DPD6, DPD10, DTE3, DTE6, DTE9, DBA2, DBA4); DPD= Pagar Dewa; TE = Tanjung Eran, BA= Batu Ampar)

Based on the electrophoresis results, the length of the DNA sequence was determined to be approximately ±400 bp. The PCR products displaying clear (clean) bands were subjected to sequencing to obtain the mycorrhizal DNA sequences. The resulting sequences were subsequently cleaned and processed using BioEdit and MEGA 11 software. Of the 15 samples analysed, eight sequence bands were faint, thin, or double, indicating poor sequence quality. The remaining seven sequence bands (DTE2, DPD2, DBA8, DBA3, DTE9, DBA2, DPD8) were bright, thick, and single, demonstrating higher quality. However, only three samples (DTE2, DPD2, and DBA8) produced sequences that could be successfully read by the DNA sequencer. High-quality sequences—those represented by bright, single bands—were then used for the subsequent data analysis stage.

Figure 2 illustrates that DNA sequences are generally detectable when the DNA bands are bright, thick, and single. Damaged DNA bands tend to be shorter in length, preventing amplification of the target DNA. After obtaining the nucleotide sequence results, the sequences were compared with those available in the GenBank database to determine the degree of similarity between the samples and known sequences. Of the 15 samples tested, only seven produced high-quality PCR products, which were subsequently sent to 1st BASE PT. Genetika Science Indonesia for sequencing. PCR failure is likely attributable to impurities present during DNA extraction, such as polysaccharides, polyphenols, residual salts, and proteins. These contaminants may inhibit the PCR process, leading to incomplete amplification or the appearance of background smears. The presence of salts in the DNA preparation can interfere with gel migration, resulting in fuzzy bands or the formation of secondary structures. In root tissues, polysaccharides and polyphenols pose particular challenges due to their abundance and reactivity. Another possible cause of amplification issues is non-specific amplification. Factors such as low annealing temperature, poor primer specificity, or excessive cycle numbers may cause primers to bind to non-target sites, generating multiple amplicons (double bands) rather than a single, correct one. Multiple DNA templates, including microbial DNA or allelic variation, may also contribute to non-specific amplification (Thompson *et al*., 2002). Inappropriate primer concentrations—either too low or too high—can result in failed amplification or primer dimer formation, the latter appearing as additional non-specific bands adjacent to the main band (Padmalatha & Prasad, 2006).

Mycorrhizal fungi may infect one or multiple plant species, and conversely, a single plant species may host different types of mycorrhiza (Mutakim *et al*., 2022). This biological complexity can introduce bias into the PCR process, as plant roots often contain DNA from multiple symbiotic fungal partners.

The subsequent stage of PCR analysis involved determining nucleotide sequences using sequencing methods. Among the seven sequenced samples, only one isolate produced a nucleotide sequence that could be accurately read by the DNA sequencer. Four samples yielded DNA bands shorter than 400 bp, preventing accurate size determination. The results of the species sequence comparison with the GenBank NCBI database are presented in Table 3.

Table 3: *Species Results in NCBI GenBank*

Number	Sample Code	BLASTn		
		Species	GenBank Code	Percent of Identity (%)
1	DTE2	Azadirachta indica	OL536374.1	93.47
2	DPD2	Basidiomycota sp.	MT645155.1	91.82
		Uncultured fungus	MF568791.1	90.70
		Delicatula integrella	MN906231.1	90.58
		Delicatula integrella	OR824591.1	90.57
		Uncultured fungus	MT672409.1	90.53
		Uncultured fungus	MH636718.1	90.50
3	DBA8	Azadirachta indica	JX856538.1	91.41
4	DBA3	-	-	-
5	DTE9	-	-	-
6	DBA2	-	-	-
7	DPD8	-	-	-

Among the three sequenced samples, one—sample DPD2 from Pagar Dewa Village—was found to contain mycorrhizal DNA, suggesting an association with mycorrhizal fungi. The identification results indicated that the nucleotide sequences of this sample corresponded to species of mycorrhizal fungi. In contrast, samples DBA8 and DTE2 were identified as having nucleotide sequences belonging to the host tree species (non-mycorrhizal). This may occur when the purity of the extracted DNA is suboptimal, which can negatively affect PCR amplification and lead to the production of unwanted or non-specific amplification products. To ensure reliable amplification, DNA extracts must be free from contaminants such as proteins or residual materials from purification processes, including phenol or alcohol (Sambrook & Russel, 1989).

The detected nucleotide sequences were subsequently compared with DNA sequences in the GenBank database to determine the degree of similarity with known species. Based on BLAST results, the highest similarity level was 91.82%, corresponding to *Basidiomycota* sp., while the lowest was 90.50%, corresponding to *Uncultured fungus*. The types of mycorrhizal fungi identified based on these similarity levels comprised six taxa: *Basidiomycota* sp. (MT645155.1), *Uncultured fungus* (MF568791.1), *Delicatula integrella* (MN906231.1), *Delicatula integrella* (OR824591.1), *Uncultured fungus* (MT672409.1), and *Uncultured fungus* (MH636718.1). All species detected in GenBank have not previously been reported as being associated with *A. excelsa* trees. This is likely due to the absence of prior studies employing biomolecular techniques on *A. excelsa* root samples. The present findings contribute to enriching the GenBank database by providing new information about fungal taxa that may not yet have been recognised, thereby serving as a valuable resource for future research. Nevertheless, further studies are required to confirm the presence of these fungi within the root systems of *A. excelsa* trees. The currently unidentified fungal species associated with *A. excelsa* roots may result from limited research into the fungal symbioses of this species. It is believed that this situation arises because *A. excelsa* has not yet been incorporated into large-scale industrial forestry practices and has thus far been cultivated primarily as a native species within community-managed forests.

Among the three species detected, one is *Delicatula integrella* (MN906231.1), *Delicatula integrella* (OR824591.1), a fungus belonging to the Basidiomycota division. Generally, Basidiomycota live as saprophytes on the remains of living organisms, such as leaf litter on the ground, straw, rice, and dead tree trunks. According to Lilleskov *et al*. (2002) and Taylor *et al.* (2016), there are also types of Basidiomycota that form symbiotic relationships with plant roots, creating mycorrhizae. Meanwhile, the origins of the other two types, Basidiomycota sp. (MT645155.1), Uncultured fungus (MF568791.1), Uncultured fungus (MT672409.1), and Uncultured fungus (MH636718.1), are not yet known. Uncultured fungi are organisms that have never been successfully cultivated or cultured in a laboratory but are instead identified through metagenomics (i.e., the complete DNA of an ecosystem, such as a handful of soil, plant roots, etc.). Although they can be identified through DNA extraction, fungal cultures are not produced. Generally, the classification of uncultured fungi only reaches the kingdom level and is not more specific.

Based on the sequence results obtained, one family was detected: Agaricaceae from the order Agaricales, with the species *Delicatula integrella* (MN906231.1) and *Delicatula integrella* (OR824591.1). Khairani (2022) stated that Agaricales is one of the orders with the highest diversity found in Indonesia, consisting of six families, such as Agaricaceae, Hygrophoraceae, Marasmiaceae, Mycenaceae, Pleurotaceae, and Psathyrellaceae. Anwar & Kataren (2023) mentioned that the results of their research indicate that the *A.* excelsa tree is one of the tree species with Angiospermae seeds whose roots are associated with ectomycorrhiza, one of which belongs to the Agaricales order and the Basidiomycota division, which was also found in this study. Basidiomycota sp. (MT645155.1), Uncultured fungus (MF568791.1), Uncultured fungus (MT672409.1), and Uncultured fungus (MH636718.1) are classified as Unclassified Basidiomycota, or their types have not been definitively classified in GenBank.

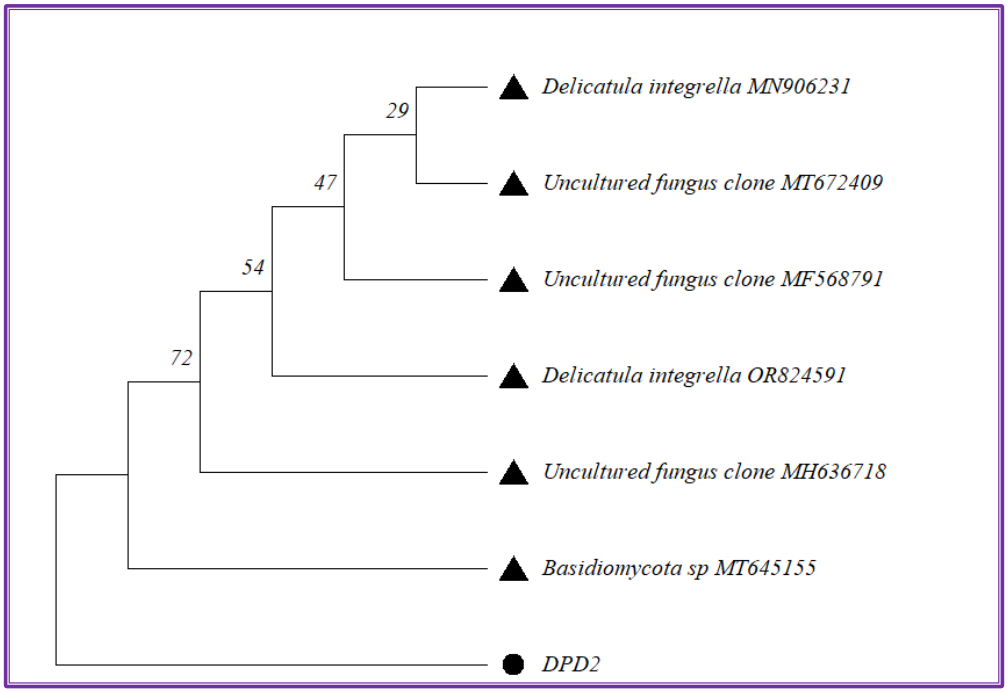

Figure 3: *Phylogenetic Tree Reconstruction of Identification Sequences in NCBI GenBank*

The results of the phylogenetic reconstruction for the sample coded DPD2 from Pagar Dewa Village, South Bengkulu Regency, using the K2P Bootstrap 1000× model, are presented in Figure 3. The phylogenetic tree was specifically reconstructed to identify the fungal sequences detected in the DPD2 sample from Pagar Dewa Village.

Based on the phylogenetic tree reconstruction, the bootstrap values for the detected species ranged from 29% to 72%. According to Sharma and Kumar (2021), bootstrap values are commonly used to evaluate the reliability of inferred species relationships obtained from different sequence alignments. The bootstrap value of 72% observed for one of the clades suggests a moderate level of support for the inferred relationships within the fungal phylogeny. Bootstrap values indicate how consistently a specific branch appears across multiple resampled datasets, reflecting the confidence in the inferred grouping (Felsenstein, 1985). Values exceeding 70% generally indicate a strong likelihood of clade validity; however, such support is not definitive and must be interpreted with caution (Hillis & Bull, 1993). Given the complex evolutionary history of fungi, moderate bootstrap support highlights the need for further validation, such as the inclusion of additional genetic markers or the application of complementary phylogenetic methods, to confirm evolutionary relationships more robustly. *Delicatula integrella* (MN906231.1) and *Uncultured fungus* (MT672409.1) were found on the same branch in the phylogenetic reconstruction. Meanwhile, *Basidiomycota* sp. (MT645155.1) appeared on a branch alongside five other species. *Basidiomycota* sp. (MT645155.1) and *Uncultured fungus* (MH636718.1) were located on the same branch, with a bootstrap value of 72%, which is considered relatively strong and reliable.

The bootstrap support values ranged between 29% and 72%, reflecting varying levels of confidence in the inferred phylogenetic relationships. Branches with support values below 50% were considered inadequately resolved and were therefore excluded from further analysis. Clades exhibiting moderate support (50–69%) indicate provisional groupings, while those with bootstrap values approaching 70% may represent biologically meaningful relationships. However, these require confirmation through additional genetic loci, morphological data, or alternative phylogenetic approaches to ensure validity.

Biomolecular techniques can be effectively employed to confirm the identity of mycorrhizal fungi as symbionts within infected roots. This has been demonstrated by Ulfa *et al*. (2019), who identified 73 species of ectomycorrhizal fungi associated with dipterocarp trees in secondary tropical rainforests—each exhibiting unique genetic characteristics and belonging to 13 different families, including

Clavulinaceae and Tricholomataceae. A related study conducted by Anwar and Ketaren (2023) also reported associations between mycorrhizal fungi and *A. excelsa* trees. Ten fungal species were identified, distributed across four families: one from the Agaricaceae family, one within the order Agaricales, one within the class Agaricomycetes, and one within the division Basidiomycota.

The findings of the present study are of significant importance for enhancing the growth quality of *A. excelsa* seedlings cultivated in nutrient-deficient soils by inoculating them with suitable mycorrhizal species.

Conclusion

Based on the findings of this study, it was concluded that three species of mycorrhizal fungi were associated with *A. excelsa* trees from Pagar Dewa Village. One of these belongs to the Agaricaceae family—*Delicatula integrella* (MN906231.1) and *Delicatula integrella* (OR824591.1)—which are classified within the order Agaricales, class Agaricomycetes, and division Basidiomycota. The remaining species, *Basidiomycota* sp. (MT645155.1), *Uncultured fungus* (MF568791.1), *Uncultured fungus* (MT672409.1), and *Uncultured fungus* (MH636718.1), are categorised as unclassified Basidiomycota or could not yet be definitively classified in the GenBank database. Samples obtained from Tanjung Eran Village and Batu Ampar Village were identified as belonging to host trees rather than mycorrhizal fungi. The bootstrap values, ranging from 29% to 72%, indicate a low to moderate level of support for the inferred phylogenetic relationships among the fungal species identified on the roots of *A. excelsa*.

Conflict of Interest

The authors state there is no conflict of interest.

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