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# Analysis of Genetic Variation *of Ganoderma sp*. as an Initial Step in Controlling Palm oil Basal Stem Rot Disease

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**Abstract**

Basal stem rot (BSR) disease caused by *Ganoderma spp.*, particularly Ganoderma boninense, represents a major threat to the sustainability of Indonesia's oil palm industry, resulting in productivity losses of 50-80%. High genetic variation among pathogens constitutes a primary obstacle in developing effective control strategies. This study aimed to analyze the genetic variation in Ganoderma as a preliminary step toward controlling basal stem rot in oil palm using ITS region DNA sequencing with ITS1 and ITS4 primers. Three Ganoderma sp. isolates were used: code 1 from the Biotechnology Laboratory of Lampung University, code 2 from the Plant Pest and Disease Laboratory of Gadjah Mada University, and code 3 from an online store. DNA extraction was performed using the TIANGEN Plant Genomic DNA Kit; PCR amplification with ITS1-ITS4 primers; and the PCR products were sequenced and analyzed using BioEdit and NCBI BLAST. Results showed that isolates code 1 and code 2 were identified as *Ganoderma boninense*, while isolate code 3 was contaminated with *Fusarium sp*. Alignment analysis using BioEdit confirmed extremely high genetic variation between the two *G. boninense* isolates with extensive nucleotide polymorphisms. This significant intraspecific diversity indicates the complexity of basal stem rot pathogen populations and emphasizes the importance of molecular approaches for accurate characterization. These findings provide a scientific foundation for developing more effective and adaptive control strategies and support oil palm resistance breeding programs based on representative pathogen genetic characteristics.

**Keywords:** Basal Stem Rot, *Ganoderma boninense*, Genetic Variation, ITS rDNA, Oil Palm

## 1. Introduction

Basal stem rot (BSR) disease, caused by *Ganoderma spp.*, especially *Ganoderma boninense*, is the most destructive disease of oil palms in Southeast Asia and has become a major threat to the sustainability of the Indonesian palm oil industry (Susanto et al., 2013; Zakaria, 2023). The impacts of this disease include a decrease in productivity of 50-80%, plant death at various planting ages, increased replanting costs, and a shortening of the economic life of the plantation by up to 50% (Purnamasari et al., 2012 Istiqomah et al., 2024) The complexity of the pathogen's life cycle, its ability to survive for a long time in soil lignocellulosic residues, and pathogenic variations between isolates make a single control strategy less effective and encourage the need for an integrated approach based on an understanding of the biology and genetics of the pathogen (Minarsih et al., 2016) ; (Widiastuti et al., 2020).

One of the root problems in controlling BPB is the high genetic variation of *Ganoderma sp*. in the field, which affects the virulence spectrum, ecological adaptation, and responses to biological and chemical control agents (Minarsih et al., 2016). The heterothallic mating system in *Ganoderma* opens up opportunities for genetic recombination, resulting in diversity of pathogen genotypes and phenotypes between locations and over time (Zhou et al., 2024; Nguyen et al., 2023). Taxonomically, morphologically based identification is often inadequate due to overlapping characters, so molecular approaches have become the standard for diagnosis and diversity studies (Purnamasari et al., 2012). ITS *rDNA* markers are the primary markers for species delineation, while random genomic markers such as RAPD and AFLP allow mapping of intra-species diversity, population structure, and gene

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flow (Pawlik et al., 2015).

*The Internal Transcribed Spacer* (ITS) region of fungal *ribosomal DNA* (rDNA) is a highly variable sequence and is highly important for differentiating fungal species through *PCR analysis.* (Fryssouli et al., 2020) . Primers ITS1 and ITS4 have become the most widely accepted universal primers for ITS sequence amplification in fungi, including the genus *Ganoderma.* (Jargalmaa et al., 2017; Gunnels et al., 2020). This primer combination can amplify the entire ITS region (ITS1, 5.8S, and ITS2) with a high success rate across various *Ganoderma sp*ecies and provides reliable phylogenetic information for species identification (Adotey et al., 2024).

Recent developments demonstrate a strengthening of the molecular basis for integrated upstream-downstream control. An ITS meta-analysis study corrected the taxonomy and clarified global *Ganoderma diversity* by identifying five major lineages (Clade AE) based on a comprehensive phylogenetic analysis (Fryssouli et al., 2020). Genomic research *on G. boninense* has provided insights into virulence factors, cell wall- and lignin-degrading enzymes, and pathogenicity signaling systems (Chen et al., 2012; Utomo et al., 2018). A multi-omics (proteomics–metabolomics) approach to infected oil palms revealed differential biochemical responses in resistant versus susceptible genotypes (Othman et al., 2024; Ahmad et al., 2024). *In silico QTL* studies and diagnostic marker validation have accelerated the screening of tolerant planting materials (Daval et al., 2021). (Permatasari et al., 2023) . Efficient *DNA* extraction protocols have been developed specifically for *Ganoderma sp*p., enabling high-quality *DNA isolation for genome sequencing and phylogenetic analysis* (Nagappan et al., 2018; Nagappan et al., 2021).

Sequence validation using BLAST against the NCBI database enables accurate species identification and detection of misidentifications in GenBank (Jargalmaa et al., 2017; Gunnels et al., 2020). BioEdit is one of the most commonly used programs in molecular biology studies. This program was originally developed as a biological sequence alignment editor for Windows only. BioEdit offers many features for sequence alignment, including easy manual alignment, separate-window view, user-definable colors, information-based coloring, and automatic integration with other programs such as ClustalW and Blast. However, in recent years, this software has evolved rapidly with the integration of many other features and functions as well as useful molecular tools for molecular biologists, such as various manual alignment modes, plasmid drawing and annotation, restriction mapping, and many more. BioEdit has become one of the most widely used programs in the field of molecular biology with its multi-purpose tools in molecular biology (Hall, 2011) .

With this background, the analysis of genetic variation *of Ganoderma sp*. as an initial step in controlling BPB is positioned as a scientific foundation for: (i) mapping the diversity and structure of pathogen populations across landscapes; (ii) adjusting integrated ecological-genetic-based control strategies; (iii) accelerating resistance breeding through representative pathogen targets; and (iv) strengthening early detection and molecular surveillance systems in plantations (Siddiqui et al., 2021; Hayati et al., 2021). This study focuses on the genetic characterization *of Ganoderma sp*. causing BPB in oil palm plantations using ITS region *DNA sequencing* with ITS1 and ITS4 primers, which will then be analyzed using NCBI BLAST for species identification (Istifadah et al., 2024) and for genetic variation analysis using BioEdit, as a basis for designing effective, adaptive, and sustainable control.

## 2. Material and Methods

Research was carried out at the CV Laboratory. Agribiotech Yogyakarta, coordinates 7°46'05.3"S 110°21'11.7"E, ±120 m above sea level, from November to December 2024. The tools used were digital scales, tweezers, mortar and pestle, microcentrifuge, micropipette, blue tip, yellow tip, white tip, and a 1.5 mL tube. The materials used were one pure isolate of *Ganoderma sp*. fungus from the collection of the Biotechnology Laboratory, Faculty of Agriculture, University of Lampung (code 1), one pure isolate of *Ganoderma sp*. fungus from the collection of the Plant Pests and Diseases Laboratory, Faculty of Agriculture, Gadjah Mada University ( code 2), and one pure isolate of *Ganoderma sp*. purchased from an online store ( code 3 ). The research implementation included DNA extraction from *Ganoderma sp*. fungus, conducting Polymerase Chain Reaction (PCR) using ITS1 and ITS4 primers, and sequencing the PCR products with ITS1 and ITS4 primers.

DNA extraction of *Ganoderma sp*. fungus using the Plant Genomic DNA Kit from TIANGEN China. The extraction was carried out according to the Kit protocol, with some modifications. In brief, the DNA extraction process is as follows: Take the fungal mycelium grown in a petri dish or test tube containing Potato Dextrose Agar (PDA) media. Place the fungal mycelium in a mortar and add 700 µL of GP1 solution. The fungal mycelium was ground with a pestle until smooth. The solution was transferred to a 1.5 mL tube and heated in a water bath (temperature 65 °C, 20 minutes). The tube was shaken twice. Add 700 µL of chloroform and shake the tube until the solution is homogeneous. The tube was centrifuged at 100 °C. 11,000 rpm for 10 minutes. The supernatant was transferred to a new 1.5 mL tube. Add 700 µL of GP2 solution and shake to homogenize. All solutions were transferred to a Spin Column CB3 in a 2.0 mL Collection tube. The Spin Column was centrifuged at 11,000 rpm for 1 minute and 30 seconds. The filtrate was discarded. Place the Spin Column back into the Collection tube.

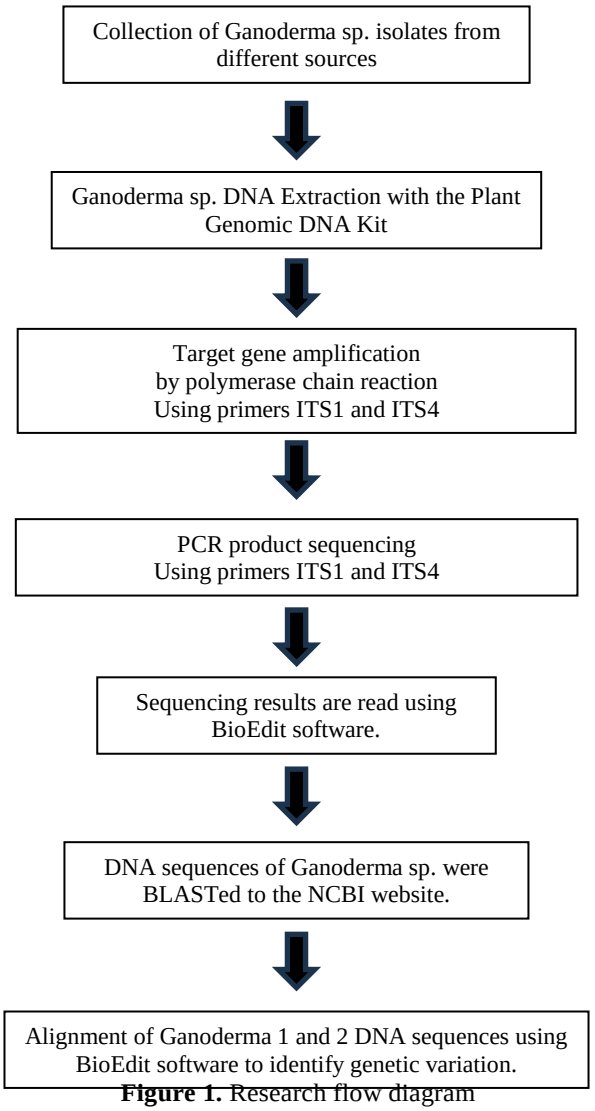


**Figure 1.** Research flow diagram

Add 500 µL of GD solution. The Spin Column was centrifuged at 11,000 rpm for 1 minute and 30 seconds. The filtrate was discarded. Place the Spin Column back into the Collection tube. Add 600 µL of PW solution. The Spin Column was centrifuged at 11,000 rpm for 1 minute and 30 seconds. The filtrate was discarded. Repeat the steps once more. The Spin Column was returned to the Collection tube. Then, it was centrifuged at 11,000 rpm for 4 minutes. The filtrate is discarded. Open the Spin Column cap at room temperature for 5 minutes to allow the membrane to dry. Place the Spin Column into a new 1.5 mL tube and add 60 µL of TE buffer to the membrane. The Spin Column is left at room temperature for 5 minutes to allow the TE buffer to penetrate the membrane. The Spin Column is centrifuged at 11,000 rpm for 4 minutes to allow the DNA to pass through the membrane and into the 1.5 mL tube. If the DNA is not immediately PCR-treated, it can be stored at -20 °C in a freezer.

DNA samples code 1, 2, and 3 were PCR amplified using 2x PCR master mix Solution (i-StarTaq™) from iNtRON Biotechnology. DNA amplification using PCR master mix and ITS1 and ITS4 primers with the miniPCR mini8 machine. The PCR machine program is as follows: 95 °C (1 time, 5 minutes); 40 cycles: 95 °C (1 minute), 53 °C (40 seconds), 72 °C (1 minute); 72 °C (10 minutes). The amplification target is 650 base pairs. Visualization of PCR products was carried out by 1.5% agarose gel electrophoresis in 1X TBE buffer (100 V, 30 minutes) using Mupid-2plus Advance. The electrophoresis results were viewed using a UV transilluminator and documented with a digital camera.

PCR product sequencing was performed at Genetica Science. DNA sequencing results were analyzed using BioEdit software. The DNA sequences were then BLASTed on the NCBI GenBank website to confirm the *Ganoderma sp*. species—the *Ganoderma sp*. DNA sequences were aligned in BioEdit to determine whether there was genetic variation in the studied *Ganoderma sp*.

## 3. Results and Discussion

This study used fungal isolates originating from the Biotechnology Laboratory, Faculty of Agriculture, University of Lampung (code 1), the Plant Pests and Diseases Laboratory, Faculty of Agriculture, Gadjah Mada University (code 2), and purchased from online stores (code 3), as shown in Figure 2.

DNA sequencing analysis with primers ITS1 and ITS4 showed that the two identified *Ganoderma sp*. isolates (codes 1 and 2) were *Ganoderma boninense* species, while isolate code 3 was identified as *Fusarium sp*. Therefore, isolate *Ganoderma sp*. code 3 could not be used for DNA sequence alignment using BioEdit. This finding shows that purchasing isolates from online stores may not necessarily match what is advertised. In the future, it is better to obtain fungal isolates from trusted sources. The results of the *Ganoderma sp*. sequencing, BLASTed against the NCBI website, are shown in Tables 1, 2, and 3. Species identification using the NCBI BLAST method aligns with the molecular approach, which has been proven effective for characterizing Ganoderma in tropical plantation crops (Purba et al., 2020; Hayati et al., 2020).

The ITS region is a highly informative molecular marker for identifying fungal species, including the Ganoderma complex. Research by Harnelly et al. (2022) showed that the ITS region exhibits sufficient sequence variability to distinguish species within the Ganoderma genus, with identification accuracy of 93-100% when compared with the NCBI database.

The very high genetic variation between G. boninense isolates, codes 1 and 2, indicates significant intraspecific diversity. It appears that DNA bases from sequence numbers 1 to 688 are largely dissimilar between Ganoderma 1 and 2, as aligned using BioEdit software (Figure 3). This phenomenon has been reported in various

studies of G. boninense genetic diversity in Southeast Asia (Purba et al., 2020; Wong et al., 2021; Midot et al., 2019). Phylogenetic analysis based on SNPs-ITS in 117 G. boninense isolates from Sarawak revealed seven distinct haplotypes, with the major haplotype, GbHap1, accounting for 81.2% of the population (Midot et al., 2019).

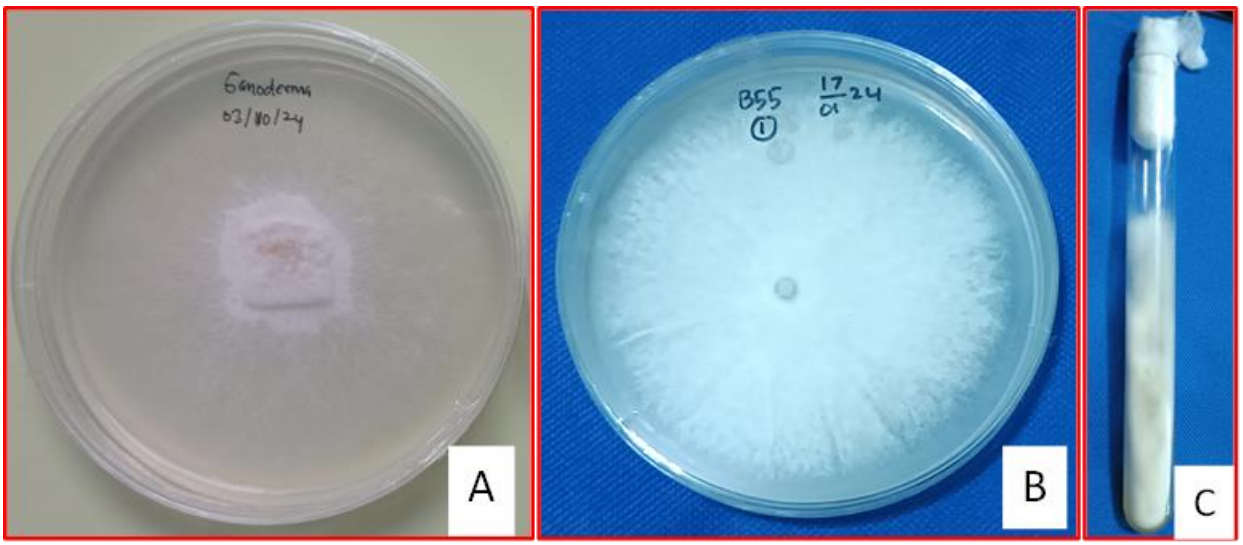


**Figure 2.** Isolates of *Ganoderma sp*. fungus originating from 3 different locations. Caption: A. Code 1; B. Code 2; C. Code 3

**Table 1.** DNA sequencing results of *Ganoderma sp*. code 1, which was blasted to the NCBI website

| **Sequences producing significant alignments** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **Per. Ident** | **Acc. Len** | **Accession** |
| *Ganoderma boninense* isolate WNBL2023042204 internal transcribed spacer 1, partial sequence | *Ganoderma boninense* | 608 | 608 | 80% | 86.93% | 613 | OR534587.1 |
| *Ganoderma boninense* isolate MGO-1 internal transcribed spacer 1, partial sequence | *Ganoderma boninense* | 569 | 569 | 82% | 85.31% | 615 | OR835528.1 |
| *Ganoderma sp*. isolate P176FB small subunit ribosomal RNA gene, partial sequence. | *Ganoderma sp*. | 569 | 569 | 95% | 82.80% | 1030 | OQ558854.1 |
| *Ganoderma boninense* isolate GB001 18S ribosomal RNA gene, partial sequence | *Ganoderma boninense* | 568 | 568 | 95% | 82.84% | 655 | KX092000.1 |

**Table 2.** DNA sequencing results of *Ganoderma sp*. code 2, which was blasted to the NCBI website

| **Sequences producing significant alignments** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **Per. Ident** | **Acc. Len** | **Accession** |
| *Ganoderma boninense* isolate K13 internal transcribed spacer 1, partial sequence | *Ganoderma boninense* | 1044 | 1044 | 89% | 98.00% | 609 | PP832821.1 |
| *Ganoderma boninense* isolate WCYZ2022111905 internal transcribed spacer 1 | *Ganoderma boninense* | 1033 | 1033 | 91% | 97.22% | 621 | OR037414.1 |
| *Ganoderma boninense* strain R.09C(F1) small subunit ribosomal RNA gene, partial sequence | *Ganoderma boninense* | 1029 | 1029 | 98% | 95.02% | 655 | PP594301.1 |
| *Ganoderma boninense* strain R.06C small subunit ribosomal RNA gene, partial sequence | *Ganoderma boninense* | 1024 | 1024 | 98% | 94.87% | 655 | PP594299.1 |

**Table 3.** DNA sequencing results of *Ganoderma sp*. code 3, which was blasted to the NCBI website

| Sequences producing significant alignments | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **Per. Ident** | **Acc. Len** | **Accession** |
| *Fusarium sp*. isolate F3Fp small subunit ribosomal RNA gene, partial sequence. | *Fusarium sp.* | 1027 | 1027 | 99% | 99.64% | 577 | PQ084739.1 |
| *Fusarium sp*. BM3 18S ribosomal RNA genes, partial sequence | *Fusarium sp.* | 1026 | 1026 | 99% | 99.47% | 564 | KJ567458.1 |
| *Fusarium sp*. isolate p20 small subunit ribosomal RNA gene, partial sequence. | *Fusarium sp.* | 1026 | 1026 | 99% | 99.64% | 560 | PP549631.1 |
| *Fusarium sp*. isolate LZ-5 small subunit ribosomal RNA gene, partial sequence | *Fusarium sp.* | 1022 | 1022 | 99% | 99.47% | 562 | MW369593.1 |

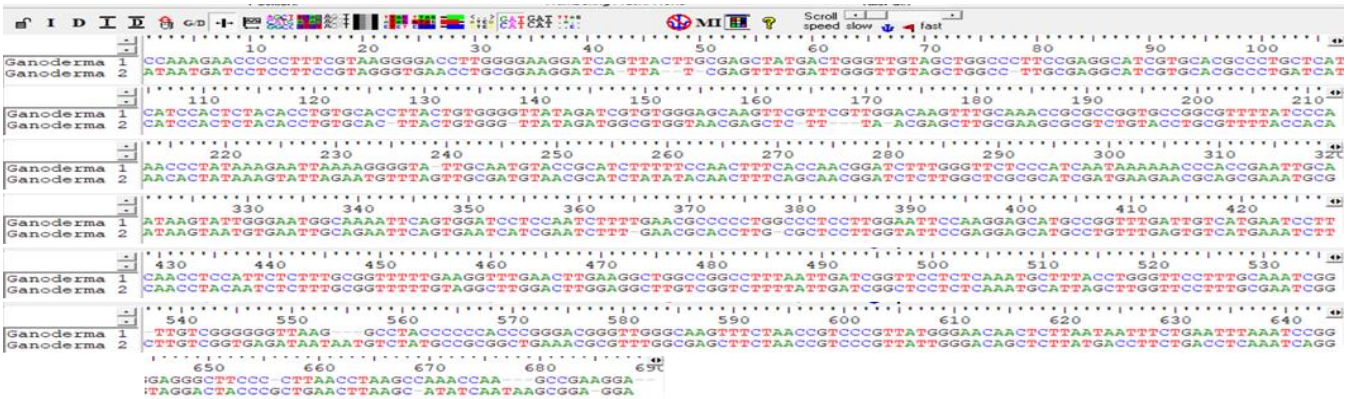


**Figure 3.** Alignment of DNA sequences of *Ganoderma sp*. codes 1 and 2 using BioEdit to determine the presence of genetic variation.

The high genetic diversity of *G. boninense* has important implications for controlling basal stem rot (BPB) in oil palm. A comprehensive study by (2021) in Malaysia and Indonesia found that *G. boninense populations* exhibit high levels of gene flow but no detectable genetic structure. This genetic diversity is also associated with varying levels of aggressiveness among isolates, with some exhibiting a Disease Severity Index (DSI) of up to 97.8% (Khoo & Chong, 2023).

## 4. Conclusion

Analysis of the genetic variation in *Ganoderma sp*. revealed a high level of genetic diversity between isolates coded 1 and 2. This significant intraspecific variation highlights the complexity of the BPB pathogen population. These findings provide a scientific foundation for developing more effective and adaptive BPB control strategies and support oil palm resistance breeding programs based on representative pathogen genetic characteristics.

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