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Characterization of *Fusarium oxysporum* f.sp. Cubense Caused Fusarium Wilt in Donomulyo, Malang Regency, Indonesia

Akhmad Rizal Oktafian[1], Irisa Trianti[2], Eriyanto Yusnawan[3], and Luqman Qurata Aini[2♥]

[1]Plant Pathology Study Program, Faculty of Agriculture, Brawijaya University, Malang, Indonesia
[2]Department of Pest and Disease, Faculty of Agriculture, Brawijaya University, Malang, Indonesia
[3]Research Center for Food Crops, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), Bogor, Indonesia
♥Corresponding author email: luqman.fp@ub.ac.id

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Abstract. *Fusarium oxysporum* f. sp. *cubense* (Foc) is a soil-borne fungal pathogen responsible for fusarium wilt, which constitutes one of the major constraints in banana cultivation. Information on the occurrence and molecular characteristics of Foc in Malang Regency, one of the banana-producing areas in East Java, Indonesia, remains limited, hindering effective disease surveillance and management. This study was conducted to isolate and characterize fungal isolates associated with fusarium wilt disease in banana plants using an integrated approach combining morphological characterization, pathogenicity testing, PCR amplification, ITS sequence analysis, and phylogenetic analysis. Three fungal isolates were recovered from symptomatic banana plants collected in Donomulyo, Malang Regency, and one representative isolate (DNM) was selected for molecular characterization based on its morphological similarity to the other isolates. Morphological observations revealed characteristics typical of *Fusarium oxysporum*. ITS sequence analysis showed 100% query coverage and 100% sequence identity with reference Foc sequences available in GenBank, while phylogenetic analysis clustered the DNM isolate with reference Foc isolates with 94% bootstrap support. Pathogenicity assays demonstrated that the isolate induced typical fusarium wilt symptoms, resulting in an LDI of 16.39% at 14 DAI and an RDI of 41.67% at 45 DAI. These findings provide baseline information on putative Foc isolates in Malang Regency, particularly in the Donomulyo area, and establish an integrated characterization framework to support disease surveillance, early detection, and the development of integrated management strategies for fusarium wilt in banana production systems.

Keywords: banana plant; fusarium wilt; Foc; PCR amplification; soil-borne disease

1. Introduction

Banana (*Musa* spp.) is a major horticultural commodity and a key contributor to national fruit production in Indonesia (Aldayanti & Ariyanti, 2023). In 2024, national banana production reached 9.60 million tons, accounting for approximately 32% of total national fruit production, making banana the most-produced fruit commodity in Indonesia (BPS, 2024). East Java Province is recognized as one of the major banana-producing regions, with Malang Regency serving as a key production center. The economic importance of the banana is further supported by its wide adaptability to diverse agroecological conditions and altitudinal ranges. However, banana production remains highly vulnerable to biotic constraints, particularly fusarium wilt disease, which poses a serious threat to sustainable banana cultivation.

Fusarium wilt is a destructive soil-borne disease caused by Foc. The pathogen can persist in soil for extended periods in the absence of a host through the formation of chlamydospores, which function as long-term survival structures (Latifah & Soedijo, 2023; Ploetz, 2015). Under favorable environmental conditions, these chlamydospores germinate and produce infective hyphae that initiate root colonization. The hyphae grow toward banana roots and penetrate primarily through wounds or natural openings in young root tissues, thereby establishing infection and facilitating subsequent vascular invasion (Dita *et al*., 2018). Yield losses caused by this disease may reach 50–100% in susceptible cultivars, thereby significantly affecting banana production (Fatma & Advinda, 2025). In Indonesia, the Australian Centre for International Agricultural Research (ACIAR)

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documented fusarium wilt incidence across 15 banana-producing provinces, including East Java, ranging from 0.08% to 100%, with an average incidence of 38.40% (Hermanto *et al.*, 2011). At the regional level, Malang Regency has experienced recurrent outbreaks, with fusarium wilt detected in eight districts during the period of 1–15 September 2018 and disease severity classified from moderate to severe (UPT PTPH, 2018). Consequently, the disease not only reduces production but also has significant economic implications for the region.

In addition to its economic impact, the management of fusarium wilt in banana is particularly challenging due to the genetic diversity and pathogenic variability of Foc. Different races and vegetative compatibility groups (VCGs) exhibit varying levels of virulence and host specificity, complicating disease diagnosis and the implementation of effective control strategies across diverse agroecosystems (Magdama *et al.*, 2020; Czislowski *et al.*, 2016). Moreover, environmental factors significantly influence pathogen survival, infection dynamics, and disease severity in the field. In regions such as Malang Regency, where agroecological conditions are highly variable, these factors may result in spatial variation in disease distribution and pathogen characteristics. Therefore, reliance solely on morphological identification may lead to misidentification or underestimation of pathogenic strains. Consequently, early detection and accurate molecular identification of Foc are essential to prevent further pathogen dissemination in this region.

Plant disease detection can generally be conducted using direct and indirect approaches. Direct methods, including molecular and serological techniques, are considered efficient for analyzing large numbers of samples (Fang & Ramasamy, 2015). Among these, Polymerase Chain Reaction (PCR) is widely applied for pathogen detection due to its high sensitivity and specificity, enabling rapid amplification of target DNA sequences and facilitating early diagnosis of plant diseases, including fusarium wilt. In the context of molecular identification of Foc, the Internal Transcribed Spacer (ITS) region of ribosomal DNA is widely recognized as the universal DNA barcode for fungal identification because it is highly conserved at the species level while retaining sufficient sequence variation to discriminate among fungal species (Aguayo *et al.*, 2017; Nozawa *et al.*, 2024). Although ITS has limited resolution for distinguishing Foc races and vegetative compatibility groups (VCGs), it provides reliable species-level identification. It is therefore appropriate for confirming the causal agent of fusarium wilt. In addition, ITS sequence analysis provides essential genetic information for phylogenetic inference and the assessment of genetic relationships among isolates, thereby improving our understanding of pathogen diversity and geographic distribution. Consequently, PCR amplification and sequencing of the ITS region represent a robust and practical approach for the molecular identification of Foc. At the same time, more discriminatory markers may be required for lineage- or race-level characterization.

Previous studies by Maryani *et al.* (2019a) have extensively investigated the diversity, taxonomy, and phylogeny of *Fusarium* species associated with banana in Indonesia. However, these studies primarily focused on the broader *Fusarium* species complex, including pathogenic, non-pathogenic, and endophytic species. They did not specifically characterize local Foc isolates associated with recent fusarium wilt outbreaks in Malang Regency through an integrated approach combining pathogenicity assays, morphological characterization, ITS-based molecular identification, and phylogenetic analysis. Recently, an increasing incidence of fusarium wilt has been reported in Donomulyo District, one of the major banana-producing areas in Malang Regency, where field observations and farmer interviews indicated that banana

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plants are affected from the early stages of growth. Despite the increasing disease incidence, the identity, pathogenicity, and molecular characteristics of local Foc isolates remain largely unknown, limiting accurate disease diagnosis and epidemiological surveillance. Characterization of local isolates is essential because Foc populations may differ in their pathogenicity, genetic composition, and geographic distribution, which can influence disease management strategies. Based on these considerations, it was hypothesized that the fusarium wilt symptoms observed in banana plants in Malang Regency were associated with local isolates of Foc. Therefore, this study aimed to isolate and characterize fungal isolates associated with fusarium wilt symptoms in banana plants using morphological characterization, ITS sequencing, phylogenetic analysis, and pathogenicity testing. The findings provide baseline information for disease surveillance, facilitate early pathogen detection, and support the development of integrated disease management strategies for sustainable banana production in Indonesia.

2. Materials and Methods

2.1 Sampling site and isolation of pathogenic fungal isolate

Pathogen sampling was conducted in a farmer-managed plantation located in Donomulyo, Malang Regency, Indonesia (8°21′14″ S, 112°25′43″ E). A total of three Cavendish banana (*Musa acuminata* AAA group) plants exhibiting characteristic symptoms of fusarium wilt were selected using a purposive sampling strategy to maximize the likelihood of isolating the causal pathogen. Each diseased plant represented one independent biological replicate, yielding a total of three biological replicates. For each biological replicate, four symptomatic stem tissue segments were aseptically placed on a single petri dish containing Potato Dextrose Agar (PDA) medium for fungal isolation. The stems were cut into 2 × 2 cm segments and surface-sterilized with 5% sodium hypochlorite for 1 min, followed by three rinses with sterile distilled water. The sterilized samples were air-dried on sterile tissue paper and aseptically inoculated onto PDA medium. Cultures were incubated for 5-7 days, after which emerging fungal colonies were subcultured onto fresh PDA to obtain pure isolates. A total of three fungal isolates, one from each symptomatic plant, were successfully obtained. Microscopic characterization of the purified isolate was conducted using slide culture techniques and examined under a light microscope at 400× magnification. Based on the similarity of colony morphology and microscopic characteristics, one representative isolate was selected for subsequent pathogenicity testing, PCR amplification, ITS sequencing, and phylogenetic analysis.

2.2 DNA extraction, PCR amplification, and sequencing of pathogenic fungal isolate

Genomic DNA extraction was performed following a modified protocol based on the method described by Maryani *et al*. (2019a), total genomic DNA was extracted from isolates grown on potato dextrose agar (PDA) for 7 days using the Presto™ Food DNA Extraction Kit (FGD) (Geneaid Biotech Ltd, Taiwan), following the manufacturer's instructions. The ITS region was amplified using ITS-specific primers, namely ITS1 (forward) 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4 (reverse) 5′-TCCTCCGCTTATTGATATGC-3′ (White et al., 1990).

The PCR amplification was performed in a total reaction volume of 50 µL containing 25 µL of 2× GoTaq® Green Master Mix (Promega, Madison, WI, USA), 5 µL each of forward and reverse primers (10 µM), 10 µL of genomic DNA template (10 ng μL^{-1}), and 5 µL of nuclease-free water. The GoTaq® Green Master Mix contained GoTaq® DNA Polymerase, Green GoTaq® Reaction Buffer (pH 8.5), 3 mM $MgCl_2$, and 400 µM of each dNTP. Amplification was carried out with an

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initial denaturation at 94°C for 4 min, followed by 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. A final extension step was carried out at 72°C for 4 min.

2.3 Phylogenetic analysis of pathogenic fungal isolate

PCR products were verified by electrophoresis on a 1.5% (w/v) agarose gel and visualized under UV illumination. Amplicons were subjected to Sanger sequencing to identify fungal species at PT. Genetic Science Indonesia. A no-template control (NTC) was included to monitor potential contamination during PCR amplification and sequencing processes. Consensus sequences were assembled using MEGA version XII and compared with related sequences available in the GenBank database using the BLASTn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Stover & Cavalcanti, 2017). Reference sequences were selected based on the highest BLASTn identity scores and query coverage, and included representative Foc isolates from different geographical origins available in the GenBank database. Multiple sequence alignment was performed using ClustalW, and phylogenetic analysis was conducted using the neighbor-joining (NJ) method based on the Kimura 2-parameter evolutionary model with 1,000 bootstrap replicates to assess branch support (Kumar *et al.*, 2024). *Fusarium solani* isolate Fs1 (PV936617.1) was used as an outgroup for sequence comparison.

2.4 Pathogenicity assay of pathogenic fungal isolate on banana seedlings

A pathogenicity assay was conducted to confirm the ability of the isolated Foc to induce disease symptoms in banana plants. Foc isolates were grown in Potato Dextrose Broth (PDB) and incubated at 27°C for 7 days to obtain adequate fungal biomass. Inoculation was performed according to the method described by Zhang *et al.* (2021), whereby banana roots were gently wounded using a sterile scalpel to enhance pathogen entry. The wounded roots were subsequently immersed in 30 mL of a Foc spore suspension for 30 min. Each treatment consisted of three biological replicates, with one banana seedling representing one experimental unit in each replicate. Disease development was assessed based on the evaluation of external and internal symptoms at 14 and 45 days after inoculation (DAI), respectively. External disease severity was scored on a 0–4 scale according to the percentage of leaf yellowing (0 = healthy, 4 = severe wilting or plant death), whereas internal disease severity was scored on a 0–4 scale based on the percentage of rhizome necrosis (0 = no symptoms, 4 = >50% necrosis). Leaf Disease Index (LDI) and Rhizome Disease Index (RDI) were calculated using the following formula:

Disease Index (%) = [Σ (Disease score × Number of plants at each score) × 100] / (4 × Total number of plants assessed) ……(1)

2.5 Statistical analysis

Disease severity data, including LDI and RDI, were analyzed using the Man-Whitney U test at a significance level of $P > 0.05$. Statistical analyses and graphical visualization were performed using R software (version 4.6.0). Data are presented as mean ± standard deviation (SD), with individual biological replicates displayed as dot plots.

3. Results and Discussion

3.1 Symptoms of fusarium wilt disease in the banana plant

Banana plants exhibiting symptoms of fusarium wilt were sampled from banana plantations in Donomulyo, Malang Regency. This area is recognized as an endemic region for fusarium wilt, with a high disease incidence (UPT PTPH, 2018). Disease assessment was conducted based on both external and internal symptoms (Figure 1). Externally, infected banana plants showed characteristic symptoms, including leaf chlorosis, initially appearing on older leaves, followed by progressive wilting and eventual

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desiccation (Figure 1a). These observations are consistent with the findings of Isbatullah *et al*. (2023), who reported that early symptoms include yellowing at the leaf margins accompanied by the formation of a yellow halo surrounding the affected areas, which subsequently turn brown and dry.

Internal symptoms were observed from transverse sections of the pseudostem, revealing vascular discoloration from white (healthy tissue) to dark brown or black (Figure 1b). Cross-sections of the corm displayed a distinct ring-like discoloration following the xylem pattern (Figure 1c–d). According to Ghag *et al*. (2015), banana plants infected by *Fusarium oxysporum* f. sp. *cubense* exhibit vascular discoloration ranging from yellow to reddish-brown. This condition indicates colonization of the vascular system by the pathogen, which disrupts water and nutrient transport, ultimately leading to systemic wilting. Overall, the observed external and internal symptoms are consistent with the typical characteristics of fusarium wilt caused by Foc.

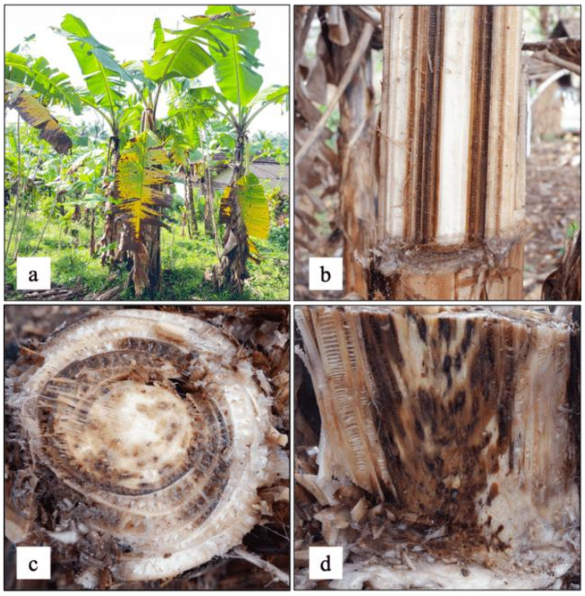

Figure 1. External and internal symptoms of fusarium wilt in banana plants. (a) External symptoms showing chlorosis of banana leaves; (b) internal symptoms characterized by brown to dark discoloration of the vascular tissues in the pseudostem; (c) ring-shaped discoloration observed in the transverse section of the pseudostem; (d) discoloration of the corm indicating advanced infection.

3.2 Isolation and morphological characterization of *Fusarium* isolates

Pseudostem tissues exhibiting these characteristic symptoms were subsequently used as the source material for pathogen isolation on PDA medium. The obtained isolate exhibited morphological characteristics typical of *Fusarium*, including white colonies with a pink to purplish reverse and a smooth, cottony texture (Ghag *et al*., 2015) (Figure 2a-b). Pink to purplish pigmentation on the reverse side of the colony is often associated with the production of naphthoquinone pigments, such as bostrycin and fusarubin, which are recognized as common phenotypic markers of Foc (Kristensen *et al*., 2021). Microscopic observations revealed the presence of oval to oblong microconidia measuring 5.0–12.0 × 2.0–3.5 µm, falcate macroconidia measuring 25.0–45.0 × 3.0–5.0 µm, and thick-walled chlamydospores formed terminally with diameters of approximately 7.0–11.0 µm (Figure 2c). These morphological and microscopic features are consistent with previous descriptions of *Fusarium oxysporum* as reported in earlier studies (Baruah *et al*., 2025; Maryani *et al*., 2019b).

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Because all three isolates exhibited similar colony morphology and microscopic characteristics, isolate DNM was selected as the representative isolate for molecular characterization, ITS sequencing, and phylogenetic analysis. However, confirmation at the forma specialis level (f. sp. *cubense*) requires further validation through molecular approaches, which are essential to enhance the accuracy of pathogen diagnosis associated with fusarium wilt.

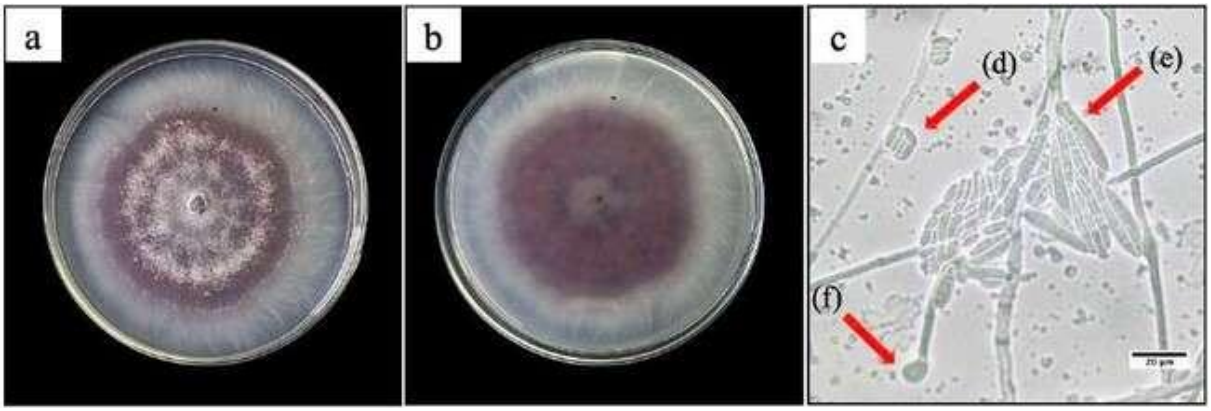

Figure 2. Morphology characteristic of the representative *Fusarium* isolate (DNM). (a) Colony morphology grown on PDA showing obverse (surface) view; (b) Reverse view of the colony; (c) Microscopic characteristics observed under a light microscope (400×), showing (d) microconidia, (e) macroconidia, and (f) chlamydospores (scale bar = 20 µm).

3.3 Molecular identification and phylogenetic analysis of isolate DNM

Agarose gel electrophoresis results demonstrated that DNA from isolate DNM was successfully amplified using universal primers ITS1 and ITS4 (Figure 3). Distinct DNA bands were observed at approximately 550–600 bp based on a 1 kb DNA marker (M), which corresponds to the expected ITS fragment size for *Fusarium* species (Pham *et al*., 2024). No amplification was detected in the negative control (NTC), indicating the absence of contamination during the PCR process.

The quality of amplification was evaluated based on the characteristics of the DNA bands, where clear, thick, and non-smearing bands indicate high-quality DNA with minimal degradation. This observation is consistent with Ramlan *et al*. (2024), who reported that high-quality DNA bands are intact and free from contamination. In contrast, the presence of smearing suggests low DNA purity. The smear patterns observed on the agarose gel indicate that the extracted DNA may contain contaminants, including proteins, organic salts, and residual RNA (Syah *et al*., 2024).

Phylogenetic analysis based on ITS sequences was conducted to determine the evolutionary relationships among fungal isolates and related species. The ITS region is widely used as a molecular marker for species-level identification and phylogenetic comparison in fungi. However, its taxonomic resolution is insufficient to reliably distinguish *F. oxysporum* formae speciales, races, or VCGs. The results of BLASTn analysis of the ITS nucleotide sequences are presented in Table 1.

The ITS sequence of isolate DNM was deposited in GenBank under accession number PZ692208.1. BLASTn analysis of the ITS sequence revealed 100% sequence identity and 100% query coverage with reference Foc sequences available in GenBank. Following sequence trimming, the aligned ITS sequence consisted of approximately 530 bp, with no nucleotide mismatches detected relative to the closest reference sequence. If two ITS sequences exhibit a similarity of ≥99%, they are highly

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likely to represent the same species (Garnica *et al*., 2016). Furthermore, the BLASTn analysis yielded an E-value of 0.0, indicating a highly significant sequence match and providing molecular evidence supporting the identification of isolate DNM as *Fusarium oxysporum*, with ITS results being consistent with Foc reference sequences available in GenBank.

The ITS sequences were subsequently subjected to phylogenetic analysis to infer the evolutionary relationships among the obtained isolate and reference *Fusarium* isolates. Phylogenetic analysis based on ITS sequences revealed that the DNM isolate clustered closely within a single clade with *Fusarium oxysporum* f. sp. *cubense* isolate R2 retrieved from the GenBank database, supported by a bootstrap value of 94%, indicating a well-supported phylogenetic relationship (Figure 4). *Fusarium solani* isolate Fs1 was used as the outgroup. These findings indicate a close evolutionary relationship between the DNM isolate and previously reported Foc reference isolates. Combined with the BLASTn results, the phylogenetic analysis provides evidence consistent with the identification of isolate DNM as *Fusarium oxysporum*, while its clustering with Foc reference isolates supports, but does not conclusively confirm, its assignment as a putative Foc. This molecular evidence complements the morphological observations, collectively providing evidence consistent with the identification of isolate DNM. However, ITS sequences are primarily suitable for species-level identification and cannot reliably distinguish Foc races or VCGs. Therefore, future studies should employ higher-resolution molecular markers, such as TEF1-α, SIX effector genes, multilocus sequence analysis, or whole-genome sequencing, to further characterize the genetic diversity and population structure of local Foc isolates (Maryani *et al*., 2019a; Le Thi *et al*., 2022).

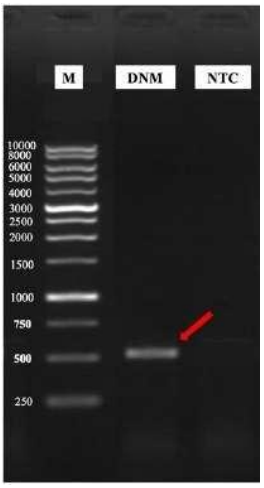

Figure 3. Pathogenic fungal isolate DNA bands amplified by PCR and visualized with agarose gel electrophoresis, where M: DNA marker; DNM: tested isolate; and NTC: No Template Control. The arrow indicates an amplified fragment (550-600 bp) corresponding to *Fusarium* sp.

Table 1. Similarity index of ITS sequences of fungal isolates based on BLASTn analysis

Isolate	Nearest Hit Species	Similarity (%)	Accession number
DNM	*Fusarium oxysporum* f.sp. *cubense*	100%	PZ692208.1

Such information will strengthen disease surveillance programs, facilitate early detection of pathogen dissemination, and support the development of more effective disease management strategies for banana production in Indonesia.

Overall, the integration of morphological characteristics, successful PCR amplification, ITS sequence analysis, and phylogenetic analysis provides complementary evidence supporting the identification of isolate DNM as *Fusarium oxysporum*. The close similarity of the ITS sequence and phylogenetic clustering with reference Foc isolates is consistent with the hypothesis that isolate DNM represents a putative Foc isolate. However, confirmation at the forma specialis level requires additional molecular markers, such as TEF1-α, SIX effector genes, or multilocus sequence analysis.

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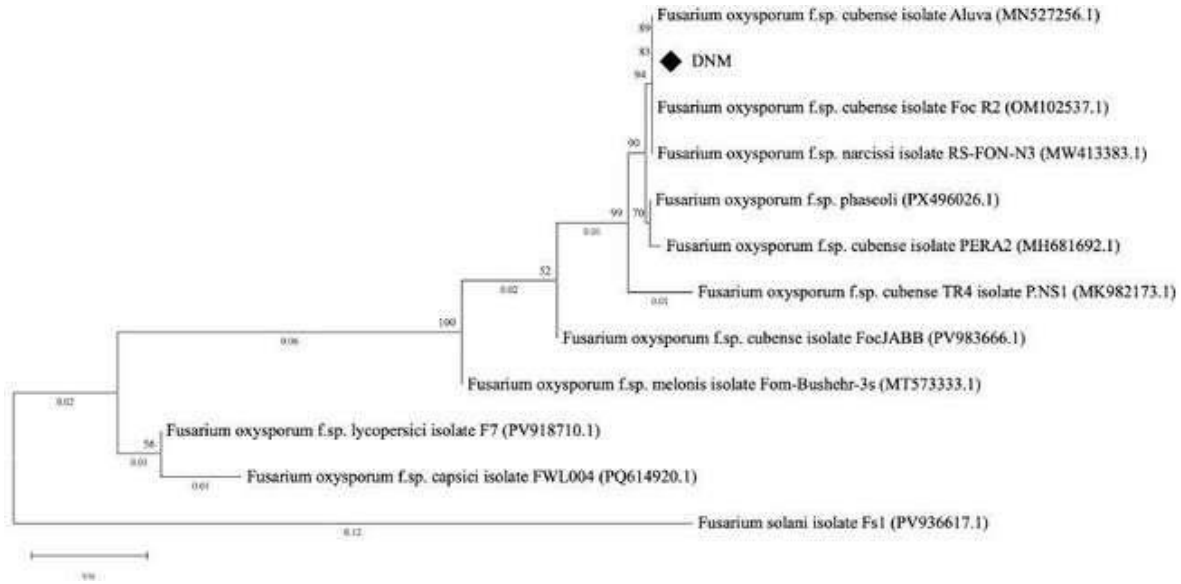

Figure 4. Phylogenetic analysis of the Foc isolate and reference sequences from GenBank (accession numbers of these sequences are indicated in parentheses next to the strain name).

3.4 Pathogenicity test of Foc on banana seedlings

The pathogenicity assay demonstrated that Foc induced typical fusarium wilt symptoms in banana plants, as evidenced by leaf chlorosis, vascular discoloration, and increased disease severity in both leaves and rhizomes (Figure 5). Control plants exhibited normal growth without any disease symptoms throughout the observation period, confirming that mechanical root wounding during inoculation did not induce disease development. In contrast, Foc-inoculated plants developed chlorosis that progressed to wilting at 14 DAI (Figure 5f), whereas vascular discoloration became evident by 45 DAI (Figure 5g–h). Disease severity reached a mean LDI of 16.39% at 14 DAI and a mean RDI of 41.67% at 45 DAI (Figure 5B-C). Although disease severity was numerically higher in Foc-inoculated plants than in the control, the differences were not statistically significant according to the Mann–Whitney U test (LDI: W = 0, P = 0.064; RDI: W = 0, P = 0.059), likely reflecting the limited number of biological replicates (n = 3). The higher RDI than LDI nevertheless indicates that internal vascular tissues were more severely affected than foliar tissues.

The observed pattern of symptom development is consistent with the infection biology of Foc. Following root penetration, the pathogen colonizes the root cortex and subsequently invades the xylem vessels, where extensive hyphal growth and microconidia production facilitate upward systemic movement through the vascular system. Progressive occlusion of xylem vessels by fungal structures, together with host-induced tyloses, gels, and phenolic deposits, restricts water transport, leading initially to leaf chlorosis and eventually to irreversible wilting (Dita *et al*., 2018; Ploetz, 2015). The substantially higher RDI than LDI further suggests that extensive vascular colonization had occurred before the complete manifestation of foliar symptoms, which is a characteristic feature of vascular wilt diseases caused by Foc.

The ability of isolate DNM to induce typical fusarium wilt symptoms, including extensive vascular discoloration and measurable disease severity, further supports its pathogenic nature. Similar findings have been reported in neighboring Vietnam, where Hung *et al*. (2018) characterized Foc isolates associated with fusarium wilt using an integrated approach combining

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morphological characterization, pathogenicity assays, molecular identification, and VCG analysis. In their study, infected banana plants exhibited characteristic fusarium wilt symptoms, including leaf yellowing and vascular discoloration, and pathogenicity was confirmed through Koch's postulates.

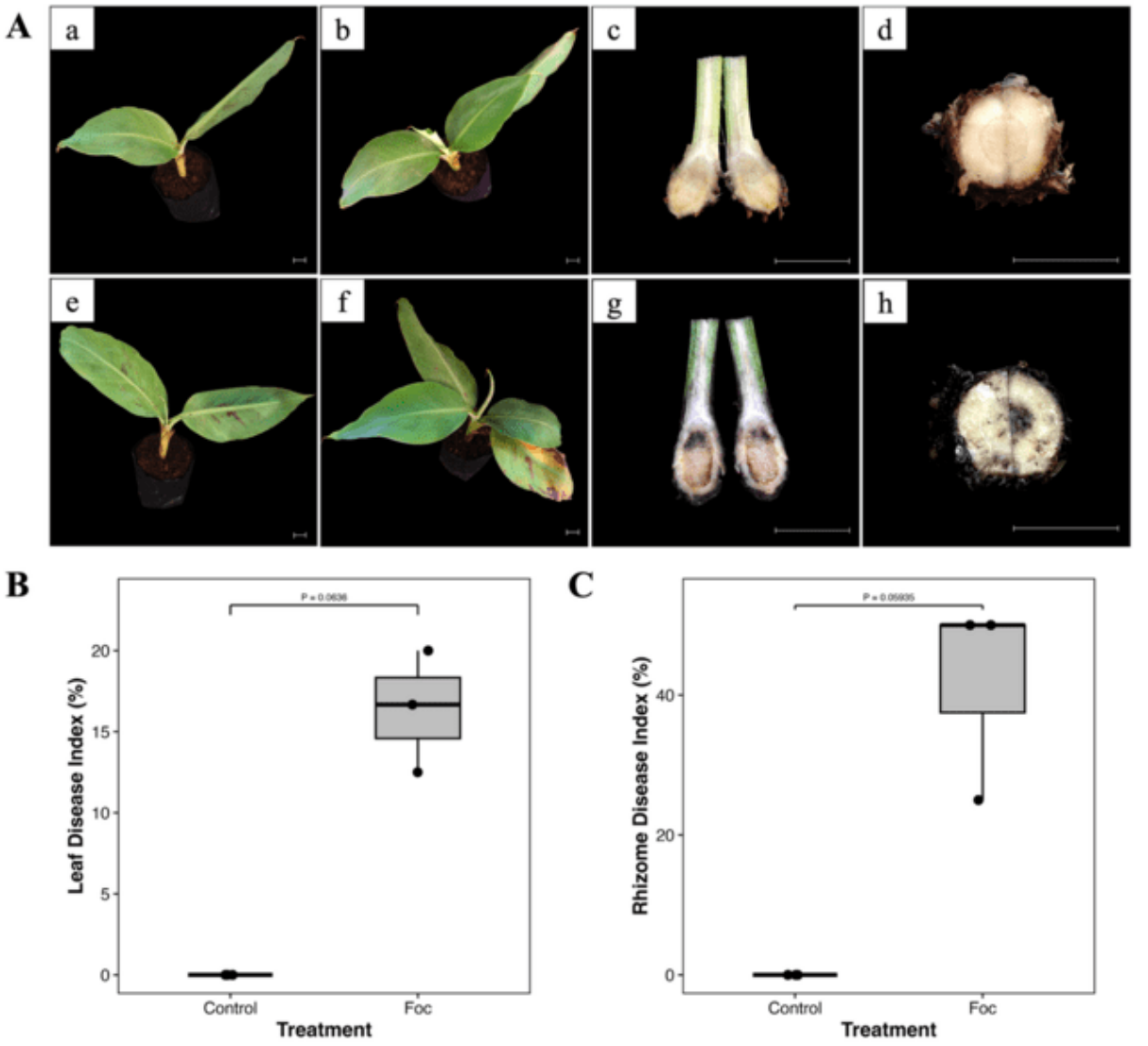

Figure 5. (A) Pathogenicity assay of Foc on banana seedlings. (a–d) Control banana plants (uninoculated with Foc); (a) Healthy banana plant prior to inoculation; (b) Control plant showing no visible symptoms of fusarium wilt at 14 DAI; (c,d) Vascular tissues and rhizome of control plants showing no discoloration at 45 DAI; (e–h) Banana plants inoculated with Foc; (e) Banana plant prior to Foc inoculation; (f) Foc-inoculated plant exhibiting typical fusarium wilt symptoms, including leaf yellowing and wilting at 14 DAI; (g,h) Vascular tissues and rhizome of Foc-inoculated plants showing pronounced discoloration at 45 DAI (scale bars: 3 cm). (B) Leaf disease index (%) of control and Foc-inoculated banana plants at 14 DAI (Mann-Whitney U test, W= 0, P = 0.0636). (C) Rhizome disease index (%) of control and Foc-inoculated banana plants at 45 DAI (Mann-Whitney U test, W = 0, P = 0.05935).

The consistency of symptom development between the two studies highlights the value of integrating morphological observations, pathogenicity assays, and molecular characterization to improve the accuracy of Foc identification

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and support disease surveillance in banana-producing regions. The pronounced vascular discoloration and systemic disease progression observed in this study share similarities with the symptom characteristics reported for Foc race 4 isolates by Guo *et al.* (2015), which exhibited more rapid xylem colonization and generally caused more extensive vascular discoloration and more severe disease symptoms than race 1 isolates. Although the physiological race of isolate DNM was not determined in the present study, the observed disease phenotype indicates that the isolate is capable of colonizing vascular tissues and inducing characteristic fusarium wilt symptoms. Collectively, these findings support the pathogenicity of isolate DNM to banana plants and demonstrate its ability to cause fusarium wilt disease following vascular colonization.

4. Limitations and Future Directions

This study has several limitations. First, molecular identification was based solely on the ITS region, which provides limited resolution for distinguishing physiological races of Foc; therefore, the physiological race of the isolate could not be determined. Second, this study included only a limited number of isolates collected from a restricted geographical area within Malang Regency, which may not fully represent the genetic diversity and population distribution of Foc in the region. Future studies should therefore incorporate multilocus phylogenetic analyses using additional molecular markers, such as *translation elongation factor* 1-α (TEF1-α), *RNA polymerase II* subunit (RPB2), and *secreted in xylem* (SIX) genes. In addition, broader epidemiological surveys involving a larger number of isolates from wider geographical areas, together with genome-based characterization, including whole-genome sequencing, are recommended to provide a more comprehensive understanding of the genetic diversity, evolutionary relationships, and pathogenic characteristics of Foc populations.

5. Conclusion

This study characterized fungal isolates associated with fusarium wilt of banana in Donomulyo, Malang Regency, using an integrated approach combining morphological characterization, molecular analysis, and pathogenicity testing. Collectively, these complementary approaches provide evidence supporting the identification of isolate DNM as *Fusarium oxysporum*, while the ITS sequence similarity and phylogenetic clustering with reference Foc isolates are consistent with its assignment as a putative Foc isolate. These findings provide baseline information to support disease surveillance, early detection, and the development of integrated disease management strategies in the study area. However, because of the limited taxonomic resolution of the ITS marker, additional molecular markers are required to confirm the identity of the isolate at the forma specialis level.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

In the preparation of this manuscript, the authors used artificial intelligence–based tools solely to assist with the paraphrasing and refinement of sentence structure in order to enhance clarity, coherence, and linguistic quality. The AI support was limited to language-related improvements and did not contribute to the generation of scientific content, data interpretation, or analytical conclusions. All critical analyses, interpretations, and evaluative judgments presented in this manuscript are entirely the original work and responsibility of the authors.

Authorship Contribution Statement

Akhmad Rizal Oktafian: conceptualization, methodology, investigation, data curation, formal analysis, writing of original draft, and visualization. Irisa Trianti: methodology, investigation, writing, review, and editing. Eriyanto Yusnawan: formal analysis, validation, data interpretation, writing, review, and editing. Luqman Qurata Aini: conceptualization, supervision, project administration, validation,

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correspondence, writing, review, and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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