

Morphological and Molecular Identification of *Fusarium oxysporum*, the Causal Agent of Wilt Disease in Strawberry Plants in Bali

Identifikasi Morfologi dan Molekuler *Fusarium oxysporum* Penyebab Penyakit Layu pada Tanaman Stroberi di Bali

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ABSTRACT

Wilt disease in strawberries caused by *Fusarium oxysporum* infection is one of important diseases in Indonesia. Although *F. oxysporum* infections in strawberries have been thoroughly researched, the morphological and molecular traits, as well as virulence levels of particular local isolates in Bali's highlands, have not been investigated. This research aimed to characterize the morphology and molecular properties of fusarium wilt disease in strawberry in Bali. Research methods used are survey and sampling, disease incidence and disease severity observation, then morphological and molecular detection. The highest disease incidence rate of 17.96% was found in Candikuning I, while the lowest incidence of 1.58% was found in Pancasari I. The highest disease severity rate of 16.28% was found in Gobleg, while the lowest was found in Kembang Merta I with 0.62%. The fungal microconidium is ovoid or oval, with 1–2 septa, while the macroconidium are sickle-shaped with a sharp edge and have three or more septa. The nucleotides of the five *F. oxysporum* isolates from Pancasari, Candikuning, Kembang Merta, Gobleg, and Wanagiri were homologous with *Fusarium* sequences in the GenBank with 100% homology. This confirms that the cause of strawberry wilt in Bali is *F. oxysporum*.

Keywords: disease incidence, disease severity, macroconidium, microconidium, sequence homology

ABSTRAK

Penyakit layu pada tanaman stroberi yang disebabkan oleh infeksi *Fusarium oxysporum* merupakan penyakit penting di Indonesia. Walaupun infeksi *F. oxysporum* pada stroberi telah banyak dilaporkan, ciri morfologi, molekuler, dan tingkat virulensi dari isolat lokal tertentu di Bali belum pernah diteliti. Penelitian ini bertujuan mengarakterisasi penyebab penyakit layu fusarium pada stroberi di Bali berdasarkan karakter morfologi dan molekuler. Metode penelitian meliputi survei dan pengambilan sampel, pengamatan insidensi dan keparahan penyakit, deteksi dan identifikasi secara morfologi dan molekuler. Insidensi penyakit tertinggi sebesar 17.96% ditemukan di Candikuning I, sedangkan paling rendah di Pancasari I sebesar 1.58%. Keparahen penyakit paling tinggi sebesar 16.28% ditemukan di Gobleg, sedangkan paling rendah di Kembang Merta I sebesar 0.62%. Mikrokonidium berbentuk ovoid atau oval, dengan 1–2 septa, sedangkan makrokonidium berbentuk sabit dengan ujung runcing dan memiliki tiga atau lebih septa. Kelima isolat *F. oxysporum* asal Pancasari, Candikuning, Kembang Merta, Gobleg, dan Wanagiri memiliki persentase identitas sekuens mencapai 100% dengan sekuens-sekuens *Fusarium* pada basis data di GenBank. Penelitian ini berhasil mengonfirmasi bahwa penyebab layu stroberi di Bali adalah *F. oxysporum*.

Kata kunci: insidensi penyakit, keparahan penyakit, makrokonidium, mikrokonidium, sekuens homologi

Strawberry cultivation has been practiced by several farmers in North Sumatra, West Sumatra, West Java, Malang, Bali, and Sulawesi. Indonesia's strawberry production fluctuated during 2017, 2018, 2019, 2020, and 2021, with the production values of 12 224, 8528, 7499, 8350, and 9859 tons, respectively (Central Bureau of Statistics 2022). Declines in strawberry production may result from various factors, including pest and pathogen infestations, suboptimal cultivation practices, and adverse climatic conditions (Frakolaki *et al.* 2026).

Strawberry wilt disease is a soil-borne disease that can cause significant production loss that may reach up to 95% (Yang *et al.* 2023). This disease occurs in the early stage growth and if left unmanaged, may spread rapidly throughout the field. *Fusarium oxysporum* is a soil-borne pathogen with a broad host range and is widely distributed across various regions of the world (Rahman *et al.* 2021), including forest ecosystems and agricultural industry (Yan *et al.* 2023). This pathogen can infect plants at various stages of vegetative and reproductive development and can survive for extended periods in infected plant residues (Zakaria 2023). This disease causes vascular wilt disease or root rot in plants (Huang *et al.* 2024). The symptoms are leaf chlorosis, stunting, changes in plant vessel coloring, and rotting which can cause death (Williamson *et al.* 2012). This pathogen poses a significant threat to plants cultivated under both field and greenhouse conditions (Din *et al.* 2020).

Previous studies have reported wilt diseases caused by *F. oxysporum* in various crops and regions. In Hawaii, *F. oxysporum* f. sp. *koae* was reported to cause wilt disease in *Acacia koa* (Edel-Hermann and Lecomte 2019). In India, *F. oxysporum* has also been reported to infect tomato plants (Murugan *et al.* 2020). In Papua, Indonesia, *F. oxysporum* was identified as the causal agent of damping-off disease in *A. mangium* (Widyastuti *et al.* 2013). In our previous study, *F. oxysporum* was reported to infect soybean plants in Bali (Suanda *et al.* 2025). More recently, *F. oxysporum* was reported to cause wilt disease in strawberry plants in Berastagi, North Sumatra (Simanjuntak and Safni 2025). However, information on the occurrence and pathogenicity of *F. oxysporum* associated with wilt disease in strawberry in Bali remains limited. Therefore, this study aimed to identify the pathogen species associated with wilt disease in strawberry in Bali and to determine the incidence and severity of the disease.

A survey was conducted in July 2025 in several strawberry-growing areas in Buleleng Regency, namely Pancasari 1, Pancasari 2, Pancasari 3, Wanagiri 1, and Gobleg 1 Villages, and in Tabanan Regency, namely Candikuning 1, Candikuning 2, Candikuning 3,

Kembang Merta 1, and Kembang Merta 2 Villages. Plants exhibiting yellowing leaves, wilting, or plant death were selected as samples. Fifteen symptomatic plants were collected from each location for pathogen isolation and identification. Observations were conducted to determine the disease incidence and severity. Disease severity scoring was measured according the criteria as described in Table 1.

Macroscopic identification was performed by observing the morphology of the pathogen growing on potato dextrose agar (PDA). The characteristics evaluated included colony color, colony shape, colony surface texture, colony margin, growth pattern, and colony coverage of the culture plate (Ma *et al.* 2021). Microscopic identification was performed using the culture slide method (Knoll *et al.* 2023).

Molecular identification of the pathogen associated with strawberry wilt disease was conducted through the following steps: DNA extraction, PCR amplification, DNA sequencing, and sequence analysis. Genomic DNA was extracted following the protocol described by Doyle and Doyle (1987). The DNA amplification process was performed on a Thermo Cycle PCR machine. The primers used are ITS1 primer (TCC GTA GGT GAA CCT GCG G) and ITS4 primer (TCC TCC GCT TAT TGA TAT GC) which amplified the ITS region within the 16S–23S rRNA (White *et al.* 1990). The raw sequence data were trimmed and assembled using ChromasPro version 1.5. The resulting sequences were subsequently analyzed using BLAST against nucleotide sequences available in the NCBI database. The data was reanalyzed by aligning the sequence via MEGA 11 program with 1000 bootstraps.

Fusarium wilt disease symptoms on strawberry plants observed in the fields are yellowish green and reddish brown leaf starting from the edges. The base of the stem turned brown and the plants stunted, wilt, and dead (Table 2; Figure 1a and 1b). This

Table 1 Scoring of severity of fusarium wilt disease in strawberry plants

Score	Category
0	0% of strawberry plants are symptomless (healthy)
1	1% < X ≤ 20% of leaves are yellow or reddish brown but not yet dry
2	21% < X ≤ 40% of leaves are dry but not yet wilted
3	41% < X ≤ 60% of plants wilt but roots have not rotted
4	61% < X ≤ 80% of plants are wilted but the roots have rotted
5	81% < X ≤ 100% plants died

Table 2 Field symptoms, disease incidence and severity of fusarium wilt infecting strawberry in Bali, Indonesia

Location	Cultivar	Symptoms variation	Disease incidence (%)	Disease severity (%)
Pancasari 1, Buleleng	Sweet Charlie	Leaves turn yellow and reddish brown, wilted	1.58	2.82
Pancasari II, Buleleng	Rosa Linda	Leaves turn yellow and reddish brown, dry and wilted	4.41	4.05
Pancasari III, Buleleng	Rosa Linda	Leaves turn yellow, dry, wilt, roots rot	16.32	7.95
Candikuning I, Tabanan	Rosa Linda	Leaves turn yellow and reddish brown, dry, wilted	17.96	4.64
Candikuning II, Tabanan	Sweet Charlie	Leaves turn yellow and wilt	2.45	3.52
Candikuning III, Tabanan	Rosa Linda	Leaves turn yellow and reddish brown, dry, wilt, roots rot	4.73	9.08
Kembang Merta I, Tabanan	Sweet Charlie	Leaves turn yellow and dry	3.08	0.62
Kembang Merta II, Tabanan	Rosa Linda	Leaves turn yellow and reddish brown, dry, wilt, roots rot, and dead	2.94	10.34
Gobleg 1, Buleleng	Rosa Linda	Leaves turn yellow, dry, wilt, roots rot, and dead	11.03	16.28
Wanagiri 1, Buleleng	Rosa Linda	Leaves turn yellow and reddish brown, dry and wilted	5.48	12.75

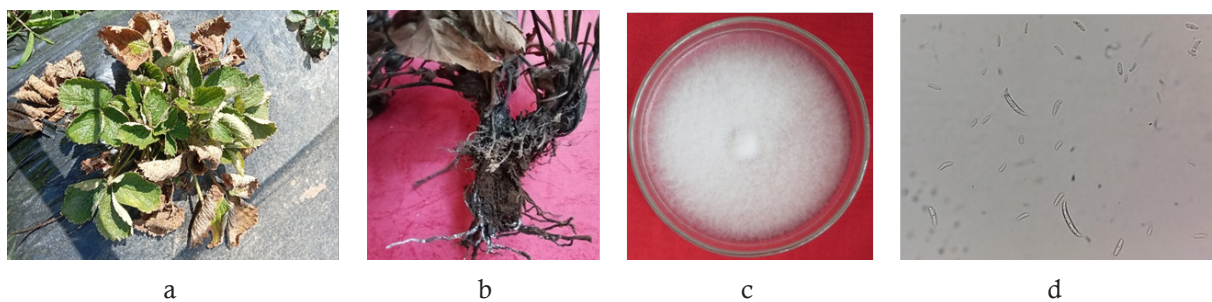


Figure 1 Symptoms on strawberry plants and morphological identification of *Fusarium*. a, the base of the stem turned brown and the plants stunted and wilt; b, dry and dead plants; c, colony morphology of white fusarium isolate on PDA media; and d, microscopic *Fusarium* spores with 400× magnification.

is in line with a research by Simanjuntak and Safni (2025) who stated that the fusarium wilt disease in strawberry plant are yellowish leaf, wilting plant, and most leaves dry. Severe symptoms causing the plant to wilt and eventually die were found in all locations, except Kembang Merta I Tabanan which only exhibit light symptoms such as yellowing leaves with drying leaf edge (Table 2). Wilt is the primary symptom of *Fusarium* wilt disease, as fungal colonization of plant root disrupts the xylem vessels responsible for water transport (Rodríguez-Bastidas *et al.* 2024).

The assessment of *Fusarium* wilt disease incidence and severity revealed varying levels of disease development among the sampled plants (Table 2). The highest disease incidence (17.96%) was recorded in Candikuning I, Tabanan, whereas the lowest incidence (1.58%) was observed in Pancasari 1, Buleleng. Disease severity was highest in Gobleg,

Buleleng, at 16.28%, while the lowest severity (0.62%) was recorded in Kembang Merta I, Tabanan. The Sweet Charlie cultivar exhibited disease incidence and severity ranging from 1.56% to 3.08% and 0.62% to 3.52%, respectively. In comparison, the Rosa Linda cultivar showed higher disease incidence and severity, ranging from 2.94% to 17.96% and 4.05% to 16.28%, respectively.

This study is the first report on the detection and identification of wilt disease in strawberry farms in Bali. Based on the macroscopic result, the colony color of the fungal isolate is white (Figure 1b). The microscopic result showed that the microconidium was ovoid or oval, with 1–2 septa, while the macroconidium was spiral or sickle with a sharp tip and has three or more septa (Figure 1c). The macroscopic and microscopic observations showed characteristics consistent with the genus *Fusarium*. Rodríguez-Bastidas *et al.* (2024)

stated that *Fusarium* spp. has cotton-like mycelium that can be white, pink, purple, or yellow in culture media. *Fusarium* spp. also has single-celled microconidium that are ovoid or oval-shaped and multi-celled macroconidium that are slightly curved with pointed tips (Figure 1c).

PCR amplification of DNA extracted from *Fusarium* isolates obtained from Pancasari, Candikuning, Kembang Merta, Gobleg, and Wanagiri using the ITS1/ITS4 primer pair produced DNA bands of approximately 500 bp (data not shown). The resulting DNA sequences from the five fungal isolates were of sufficient quality for subsequent sequence analysis. Nucleotide sequence analysis revealed 100% sequence identity between the five *F. oxysporum* isolates obtained from Pancasari, Candikuning, Kembang Merta, Gobleg, and Wanagiri and *Fusarium* sequences available in the GenBank database. The closely matching sequences originated from isolates reported in China, India, Uzbekistan, Morocco, Italy, South Africa, Malaysia, Nigeria, Colombia, and the United States (Table 3). During fungal identification using ITS sequences, sequence similarities of $\geq 97\%$ –100% with sequences in the GenBank database are generally considered sufficient for species-level identification,

corresponding to up to 3% sequence divergence (Raja *et al.* 2017). The phylogenetic analysis clustered the *Fusarium* isolates into a single clade comprising isolates from Indonesia, China, India, Uzbekistan, Morocco, Italy, South Africa, Malaysia, Nigeria, Colombia, and USA (Figure 2). Further molecular identification confirmed that all isolates of strawberry wilt disease in Bali are part of *F. oxysporum*.

This pathogen can infect plants at various stages of vegetative and reproductive development and can survive for extended periods in infected plant residues (Zakaria 2023). This disease causes vascular wilt disease or root rot in plants (Huang *et al.* 2024). This disease causes vascular wilt disease or root rot in plants (Huang *et al.* 2024). This pathogen poses a significant threat to plants cultivated under both field and greenhouse conditions (Din *et al.* 2020).

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Table 3 Nucleotide homology of *Fusarium oxysporum* isolates from Bali originating from strawberry plants to isolates from other countries available in GenBank

Isolates source	Homology				
	Pancasari	Candikuning	Gobleg	Wanagiri	Kembang Merta
Pancasari_Bali*	ID	100%	100%	100%	100%
Candikuning_Bali*	100%	ID	100%	100%	100%
Gobleg_Bali*	100%	100%	ID	100%	100%
Wanagiri_Bali*	100%	100%	100%	ID	100%
KembangMerta*	100%	100%	100%	100%	ID
OR789463_China	100%	100%	100%	100%	100%
MN856310_China	100%	100%	100%	100%	100%
PP596539_China	100%	100%	100%	100%	100%
MW264916_China	100%	100%	100%	100%	100%
PP944870_India	100%	100%	100%	100%	100%
PQ198238_Uzbekistan	100%	100%	100%	100%	100%
OL865583_Morocco	100%	100%	100%	100%	100%
KX929698_Italy	100%	100%	100%	100%	100%
MZ674474_SouthAfrica	100%	100%	100%	100%	100%
KJ767067_Malaysia	100%	100%	100%	100%	100%
MW260086_Nigeria	100%	100%	100%	100%	100%
KU671041_India	100%	100%	100%	100%	100%
OP794342_India	100%	100%	100%	100%	100%
LN828174_Colombia	100%	100%	100%	100%	100%
OR123360_USA	100%	100%	100%	100%	100%
NR_144794_C. acutatum_USA	73%	73%	73%	73%	73%

**Fusarium oxysporum* isolate from Bali, Indonesia; *Colletotrichum acutatum* from the USA was used as the out group.

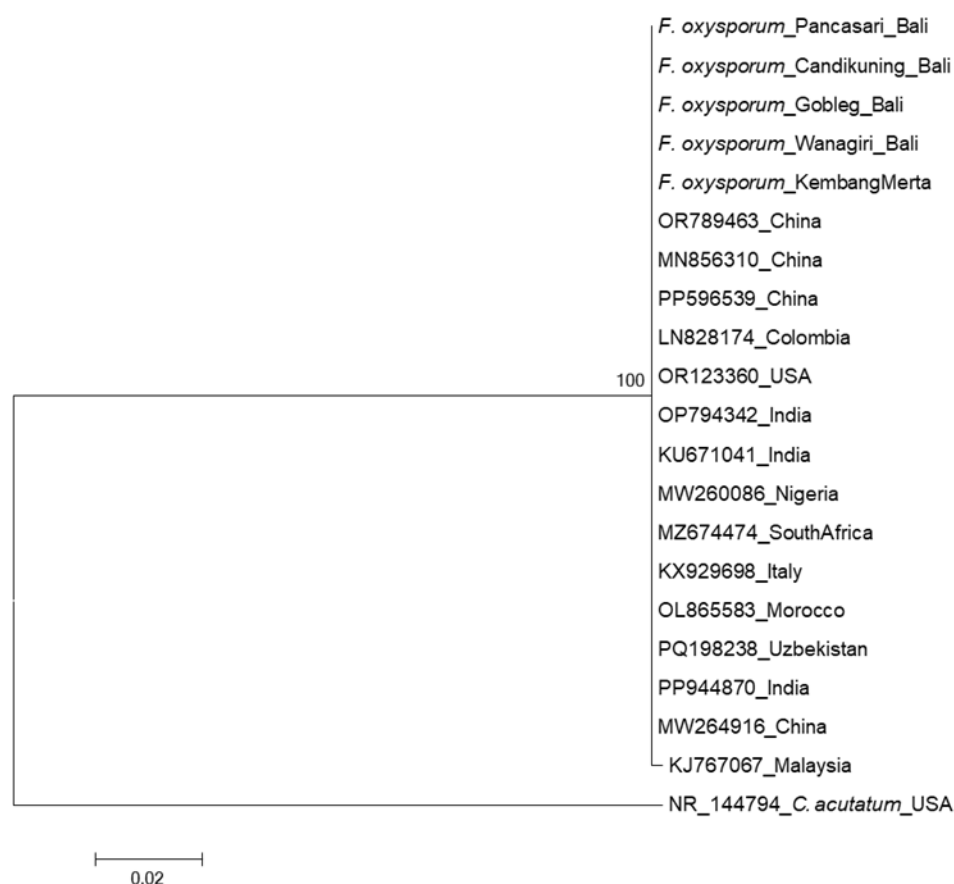


Figure 2 Phylogenetic tree of *Fusarium oxysporum* isolates obtained from strawberry plant samples from Bali, Indonesia (Pancasari, Candikuning, Gobleg, Wanagiri, Kembang Merta) constructed using the neighbor-joining method with 1000 bootstrap replicates in MEGA 11. *Colletotrichum acutatum* from the United States was used as the outgroup.

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