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Isolation and identification of pathogenic *Fusarium oxysporum* isolated from soybean leaves

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Abstract. *Suanda IW, Widnyana IK, Dharmadewi AAIM. 2025. Isolation and identification of pathogenic* Fusarium oxysporum *isolated from soybean leaves. Biodiversitas 26: 2261-2268.* Not all fungi isolated from soybeans are pathogenic. Understanding the identity and pathogenicity of the fungi found is crucial for providing appropriate control recommendations, which can significantly impact soybean farming. This research aimed to compare the pathogenicity of various fungi isolates obtained from soybean plants with withering symptoms in fields in Bali, along with morphological and molecular identification. Five fungi isolates were chosen for the pathogenicity test, and the most aggressive isolate was further tested for morphological and molecular detection with PCR by using ITS1 and ITS4 primers. The pathogenicity test was performed by inoculating fungi into healthy soybeans from Anjasmoro, Argomulyo, and Argopuro cultivars, and the symptoms were assessed five days after inoculation. Five days after inoculation, the soybean leaves turned yellowish brown, the roots were few and short on all tested fungi isolates, while the uninoculated control seed remained asymptomatic. The five isolates were different in aggressiveness; the disease area percentage ranged between 81% and 98%, depending on the isolate. The most aggressive isolates inoculated in soybeans are the JKP2 and JKP3 isolates; the highest plant proportion inoculated by these isolates showed the most severe symptoms (the diseased area was 98% or complete plant rot). The JKP2 culture grown in PDA media is generally characterized by white to greenish purple, reverse white, and fluffy. At the same time, the mycelium of JKP3 isolate is white, reverse purple in the center, and white at the margins. Phylogenetic analysis confirmed the two isolates observed to be *Fusarium oxysporum.* To our knowledge, this is the first report of *F. oxysporum* isolated from soybeans in Bali.

Keywords: Fungi, *Fusarium*, pathogenesis, PCR, soybean

INTRODUCTION

Soybeans are among the highly valued agricultural commodities due to their relatively high nutritional value. Food processed from soybeans is quite varied, such as soy milk, tempeh, tofu, soy sauce, miso, snacks, and so on; soybean demand increases, affected by the demand from industries with soybeans as base ingredients. Soybean is now even the most important national plant commodity after rice and corn in Indonesia (Rinaldi et al. 2023). Soybean productivity in Indonesia is around 1.2 tons/ha and is considered low compared to the world productivity, which reaches 1.5 tons/ha (Harsono et al. 2021). The Central Bureau of Statistics reported in 2023 that relatively large soybean imports occurred consecutively: 2,475 tons (2020), 2,489 tons (2021), 2,325 tons (2022), and 2,274 tons in 2023. Soybean production is way under the demand value, and one of the causes is disease infection in soybean crops in the field (Shea et al. 2019).

Soybean disease caused by pathogenic fungi brought by soybean seedlings are *Phomopsis* sp., *Penicillium* sp., *Aspergillus* sp., *Cercospora* sp., *Alternaria* sp., *Fusarium* sp., and *Colletotrichum dematium* var. *trucata* (Schwein) Arx. (Soesanto et al. 2020; Hosseini et al. 2023). Fungi from *Fusarium* genus are pathogenic fungi that often attack soybeans starting from the nurturing period (Olszak-Przybyś et al. 2023). Disease caused by *Fusarium* spp. showed withering symptoms in plants. As stated in a report by Tahat et al. (2021), *F. oxysporum* causes damping-off and whithering. *Fusarium oxysporum* infection starts from soybean root (Luo and Yu 2020).

F. oxysporum is one of the main pathogens because the pathogenic strain of this fungus can remain dormant for 30 years before continuing its virulence and infecting plants (Soesanto et al. 2022). According to Perincherry et al. (2019), the production loss caused by *F. oxysporum* infection reached 59%. When plants show symptoms of pathogen infection, it is already too late for control, and the plants will die. Moreover, *F. oxysporum* is a broad-spectrum pathogen because it can cause disease in almost all important agricultural crops. *F. oxysporum* is proven to be difficult to control because the conidia of *F. oxysporum* can survive on the ground for a long period, so plant rotation is not the appropriate control method (Jackson et al. 2024). According to Brown et al. (2023), *F. oxysporum* caused sudden death syndrome/SDS in soybeans. Infection begins from the root and rarely attacks the leaves.

Not all *Fusarium* isolates obtained from field-collected plants are pathogenic, meaning that not all of them can induce disease symptoms in the plants. Moreover, they may differ in aggressiveness, which is commonly defined as the quantitative variation of pathogenicity on susceptible hosts (O'Donnell et al. 2022; Olszak-Przybyś et al. 2023). Particularly about *F. oxysporum*, it is clear that isolates may

range from highly aggressive to nonpathogenic (Torres-Cruz et al. 2022). Therefore, testing the pathogenicity of the obtained *Fusarium* isolates should be an important element in identifying pathogens; it is also recommended in Koch's postulates and recent guidelines for pathogenicity testing (Bhunjun et al. 2021; Olszak-Przybyś et al. 2023). Koch's postulate test has been reported by Alwahshi et al. (2019) to determine whether *Fusarium* sp. isolates are responsible for triggering disease in *Z. hybrida* plants on the ISS. A series of Koch's Postulates tests were performed with six *F. oxysporum* strains isolated from symptomatic *Z. hybrida* tissues and two *Fusarium oxysporum* strains obtained from the kitchen in the living quarters on the ISS (Schuerger et al. 2021).

This study aimed to determine the identity and pathogenicity of *Fusarium* isolates obtained from symptomatic soybean plants grown in Bali and compare their aggressiveness. Fungal isolates from soybeans grown in this geographic region have not, so far, been subjected to similar research. *F. oxysporum* isolates tested here have been rarely studied in the context of pathogenicity for soybean seeds. Moreover, the pathogenicity of *F. oxysporum* isolates has never been tested in Bali on this crop.

MATERIALS AND METHODS

Sample collections

Fungi isolates were taken from soybeans with withering symptoms in Subak Kepaon, Pemogan Village, South Denpasar District, Denpasar City, and Subak Umabun, Angantaka Village, Abiansemal Sub-district, Badung District, Bali, Indonesia. Isolation and macroscopic and microscopic identification of pathogenic fungi were performed in Sunari Agrosaintech Laboratory, Denpasar. Pathogenicity tests, according to Koch's postulates, were performed in the Biology Education Program, Faculty of Science and Technology, Universitas PGRI Mahadewa Indonesia, Denpasar. Molecular identification and phylogenetic tree were performed with BRIN.

Pathogenicity test

Soybeans in the farm field showing disease symptoms had their pathogen-infected leaves taken as samples to be isolated in the laboratory, and from this, five pathogenic fungi isolates were obtained. The pathogenicity test of the disease-causing fungi isolates was performed through Koch's postulates test on soybean seedlings reared in polybags inside a greenhouse, which would then be compared with the symptoms in the initial soybeans. If the infected soybeans showed the same symptoms, then the pathogen inoculated is the cause of the disease in the field. The pathogenic fungi causing soybean disease were re-isolated on PDA medium and incubated for 7 days at 26-28°C. They are further purified in a new PDA medium, which results in two isolates with different colony morphology. These pathogenic fungi isolates were reassessed for pathogenicity through the second Koch's postulates test. The pathogenic fungi isolates causing the most severe damage were two isolates: JKP2 and JKP3. They were re-isolated and purified again as a stock isolate for further study.

Morphological identification

Pure cultures of soybean withering disease pathogenic fungi obtained by Koch's postulates were identified macroscopically based on their morphological characteristics and pigmentation on PDA media. Microscopic identification via microscope observation was performed by dropping a drop of methylene blue in the middle of an object glass, taking purified JKP2 and JKP3 fungi isolates aseptically with a nichrome loop, and smearing them on the methylene blue-covered object glass. The preparation was observed under the microscope with 1,000 times magnification. Microscopic identification based on the fungi mycelia was matched by the characteristic description and pictures in the Illustrated Genera of Imperfect Fungi book by Barnett and Hunter (1998). Fungal growth was recorded daily for up to 4 days and measured from the edge of the initial inoculum to the extreme area of the mycelium using four perpendicular lines drawn on the back of the Petri dish. The experiment was performed in 12 replicates, and the average value of 4 measurements made along the marked lines on the Petri dishes was recorded at 24-hour intervals. Then, the growth rate (in mm/day) was calculated and compared among the studied isolates using One-Way Analysis of Variance (ANOVA) and Tukey's post-hoc test.

Molecular identification

DNA extraction followed Doyle and Doyle's procedure (1987). As much as 0.2 g of the pathogenic fungi's mycelium sample was ground in liquid nitrogen; then, the resulting powder was put inside an Eppendorf tube. Into the tube, 500 µL CTAB buffer and 50 µL β-mercaptoethanol were added and then homogenized by vortexing. Next, to lyse the cell wall, samples were heated at 70°C for 60 minutes, and then the samples were flipped every 10 minutes to accelerate lysis. The samples were then cooled until they reached room temperature. Chloroform isoamyl alcohol (24:1) was added to 500 µL of the tube and homogenized by vortexing before centrifuging at 12,000 rpm for 15 minutes. The resulting supernatant was moved to a new Eppendorf tube by adding 500 µL of sodium acetate and then homogenized by vortex and centrifuged again at 12.000 rpm for 10 minutes. The supernatant was moved into another Eppendorf tube and supplemented with sodium acetate and isopropanol, 500 µL each. It was homogenized by vortex and centrifuged again at 12,000 rpm for 10 minutes. The tube was gently shaken to bind the DNA and then incubated at -20°C for 30 minutes. The obtained DNA strands were precipitated by centrifugation for 10 minutes. The supernatant was discarded. The pellet was washed with 70% ethanol and then centrifuged at 8,000 rpm for 5 minutes. The ethanol was discarded, and the pellet was dried. The pellet was resuspended in 50 µL TE buffer and stored at -20°C to be used further for the DNA amplification process.

DNA amplification was performed using the PCR method. The amplification used universal primers to detect the Internal Transcribed Spacer (ITS) region of the ribosomal DNA (rDNA). The primers are forward primer

ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), with the size of the amplification target being 490 bp (White et al. 1990). The DNA amplification reaction was performed in a total volume of 25 μL, which consisted of 1 μL DNA, 2.5 μL buffer 10× and Mg^{2+}, 0.5 μL dNTP 10 mM, 1 μL of each primer, 12.5 μL Taq DNA (10 units/μL), and 9.5 μL H_2O. The amplification condition was separated into several stages: pre-denaturation at 94°C for 3 minutes, followed by 30 amplification cycles where each cycle consisted of DNA strand separation/denaturation at 94°C for 1 minute, primer attachment/annealing at 45°C for 1 minute, and DNA synthesis at 72°C for 2 minutes. For the last cycle, a synthesis stage for 10 minutes was added, and the cycle ended at 4°C.

The amplification product was analyzed by Blue-LE electrophoresis in a 1% agarose gel (0.5× Tris-Borate EDTA/TBE). Electrophoresis was performed at 100 volts for 28 minutes, and the agarose gel was then incubated in a staining agent containing ethidium bromide (1%) for 15 minutes; it was then washed with H_2O for 10 minutes. The electrophoresis result was visualized by a transilluminator under ultraviolet light. The DNA strands formed as a digital camera documented the electrophoresis result.

The amplification product was sent to 1st Base (Malaysia) for nucleotide sequencing. The sequencing result was analyzed by the Basic Local Alignment Search Tool (BLAST) using the optimization program to obtain homologous DNA base sequences from the National Center for Biotechnology Information (NCBI) site. The obtained nucleotide sequence was analyzed by multiple alignment ClustalW in the Bioedit to sequence alignment editor software version 7.0.5. A homology level that is nearing 100% similarity is categorized as the same species as the sample species.

RESULTS AND DISCUSSION

Pathogenic soybean fungi exploration

The exploration result of soybean parts from Anjasmoro variety in Subak Kepaon, Pemogan Village, South Denpasar District, Denpasar City was one pathogenic isolate or the JKP1, whereas in Subak Umabun, Angantaka Village, Abiansemal District, Badung Regency, 4 isolates were obtained: JKP2, JKP3, JKP4, and JKP5 isolates (Figure 1). At the initial stage of the exploration, 23 isolates were obtained in several locations in Bali, but after isolation, contamination occurred, which resulted in only 5 isolates remaining.

Morphological identification of the soybean pathogenic fungi isolates was performed by morphological observation, such as from the culture appearance, growth rate, macroconidia, and microconidia (Table 1). Five colonies with different colors were obtained, which were white greenish, reverse brownish white (JKP1), white to greenish purple, reverse white and flocky (JKP2), white, reverse purple in the center, and white at the margins (JKP3), white with fluffy growth, reverse white and flocky (JKP4), and white to greyish red, reverse white and orange (JKP5). The variation of pathogenic isolates obtained from the two locations is possibly due to the geography of the environment where the samples were taken. This matches a study by Grabka et al. (2022), which isolated diverse endophyte fungi from soybean parts, such as the leaf, branch, and root, that live in every plant tissue. This result observed several morphological characteristic differences. The Koch postulate further tested the 5 isolates obtained. Purified pathogen isolates were nurtured in PD Broth media and incubated for 7 days.

The pathogenicity test of soybean withering disease-causing fungi

Soybeans of Anjasmoro, Argomulyo, and Argopuro cultivars aged 5 weeks (3-4 foliates) were treated with pathogenic fungal isolates. The soybeans of each treatment were smeared with carborundum 25% of the surface of the leaf underside on 2 foliates. Control treatment plants were sprayed with distilled water and soybeans without treatment. Each treatment had 12 repetitions, and all crops were covered with plastic covers. Observation was performed five days after application.

The observed characteristics included diseased area percentage, disease incidence, disease severity, and plant root condition (Table 2; Figures 2-4). Symptomatic soybean leaves were re-isolated by maintaining diseased plants on PDA media in petri dishes through incubation for seven days (t=26-28°C). The morphology of the fungal colony structure growing on the petri dishes was observed. In all treatments, the pathogens that grew had the same macroscopic structure and were present in each PDA medium. Isolates JKP2 and JKP3 were purified and grown on PD broth media through shaking and incubation for seven days. The re-isolated products were tested using Koch's postulates on 5-week-old soybean seedlings of Anjasmoro, Argomulyo, and Argopuro varieties (3-4 leaves). Five days after inoculation, all five isolates showed the same symptoms in plants after testing Koch's postulates, namely, necrotic symptoms (brown, black, or rotting tissue) appeared on soybean leaves (Figures 2 and 3), and roots became fewer and shorter compared to control plants (Figure 4). In contrast, uninoculated control seedlings remained asymptomatic (Figures 2 and 3). The percentage of disease area inoculated with *Fusarium* was significantly higher than the control (Table 2). Thus, all isolates tested could infect soybean seedlings, and all were pathogenic to this plant.

The 5 isolates differed in their aggressiveness; the disease percentage ranged from 81% to 98% for the three soybean cultivars, depending on the isolate (Table 2). The most aggressive isolates inoculated on soybean were *Fusarium* isolates JKP2 and JKP3 because the plants with the highest proportion inoculated by these isolates showed the most severe symptoms (disease area percentage of 98% or complete plant rot). Meanwhile, *F. oxysporum* isolates JKP1, JKP4, and JKP5 were the least aggressive because the disease area percentage averaged 81-87% (Table 2). The results of this study correlated with the incidence and severity of the disease. The higher the percentage of diseased areas, the higher the incidence and severity of the disease. Therefore, further morphological and molecular identification was

carried out for *Fusarium* isolates JKP2 and JKP3.

Morphological identification

Based on cultural and morphological characteristics such as growth rate, length and width of macroconidia, basal cell shape, number of septa in macroconidia, and presence or absence of microconidia in the culture, five isolates tested were tentatively classified into the same group (Table 1). The culture of isolates belonging to the first group grown on PDA medium was generally characterized by abundant white to pale purple cottony mycelium and dark purple below the surface. The average growth rate of these cultures ranged between 4.89 and 5.84 mm/day. The macroconidia were relatively slender, averaging 35.58-37.31 μm in length, thin-walled, and had 2-5 septa. Their average width ranged between 3.35 and 3.67 μm (Table 1). Microconidia were also observed in the cultures of isolates 1-5; these isolates were oval or elliptical without septa inside. The morphology of isolates JKP2 and JKP3 is shown in Figure 5.

Table 1. Characteristics of five *Fusarium* isolates, including description of culture appearance and its average growth rate (±standard deviation) based on measurements taken after 4 days of growth. Macroconidia measurements include their average (±standard deviation), length, width and the number of septa. Table also contains information on the presence (+) of foot on macroconidia and of microconidia in the culture

Isolate source	Isolates	Culture appearance	Growth rate (mm/day)	Macroconidia				Micro-conidia
				Length (πm)	Width (πm)	Septa number	Foot	
Subak Kepaon, Pemogan Village, South Denpasar District, Denpasar City	JKP1	White greenish, reverse brownish white	5.54±0.04b*	36.37±8.32b*	3.35±0.92a*	2-5	+	+
Subak Umabun, Angantaka Village, Abiansemal District, Badung Regency	JKP2	White to greenish purple, reverse white and flocky	5.76±0.58b	35.97±9.26a	3.52±0.28c	2-5	+	+
	JKP3	White, reverse purple in the centre and white at the margins	5.84±0.27b	37.31±8.45c	3.43±0.80b	2-5	+	+
	JKP4	White with fluffy growth, reverse white and flocky	4.97±0.06a	36.42±9.82b	3.55±0.98c	2-5	+	+
	JKP5	White to greyish red, reverse white and orange	4.89±0.68a	35.58±8.53a	3.67±0.62d	2-5	+	+

Note: *: Different lowercase letters in the same column indicate a significant difference (p<0.05), according to Tukey's post-hoc test

Table 2. Effects of inoculation with five *Fusarium* isolates on diseased area percentage, disease incidence, and disease severity of three soybean cultivars

Isolates	cv. Argomulyo			cv. Anjasmoro			cv. Argopuro		
	Disease area percentage (%)	Disease incidence (%)	Disease severity (%)	Disease area percentage (%)	Disease incidence (%)	Disease severity (%)	Disease area percentage (%)	Disease incidence (%)	Disease severity (%)
Control	0a*	0a*	0a*	0a*	0a*	0a*	0a*	0a*	0a*
JKP1	85b	85b	71.8b	81b	80b	68.3b	87b	85b	72.5b
JKP2	98b	100b	89.5b	92b	95b	74.7b	96b	100b	83.9b
JKP3	98b	100b	87.6b	94b	100b	87.2b	98b	100b	84.1b
JKP4	85b	84b	70.1b	81b	84b	74.5b	86b	88b	72.2b
JKP5	85b	86b	71.3b	85b	86b	73.1b	87b	86b	71.6b

Note: *: The presence of different lowercase numbers in the same column indicates a significant difference (p<0.05) according to pairwise significance tests performed after Kruskal-Wallis test

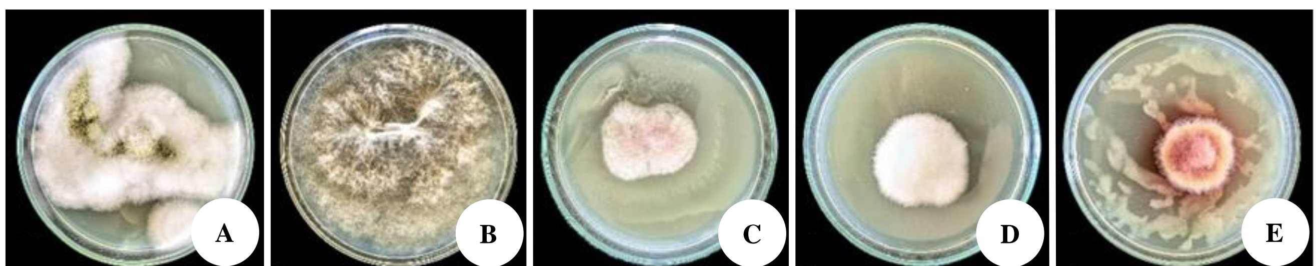

Figure 1. Pathogenic fungi isolated from diseased soybean plants (5 isolates): A. JKP1; B. JKP2; C. JKP3; D. JKP4; E. JKP5

Figure 2. Soybean plants after the Koch's postulates test was carried out using 5 isolates, namely: P1: JKP1; P2: JKP2; P3: JKP3; P4: JKP4; P5: JKP5; and C: control (without treatment) were used as a comparison

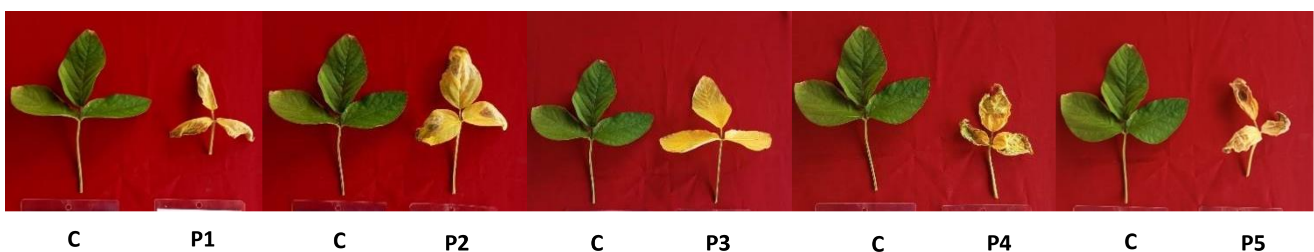

Figure 3. Soybean plant leaves after the Koch's postulates test was carried out using 5 isolates, namely: P1: JKP1; P2: JKP2; P3: JKP3; P4: JKP4; P5: JKP5; and C: control (without treatment) were used as a comparison

Figure 4. Soybean plant roots after the Koch's postulates test was carried out using 5 isolates, namely: P1: JKP1; P2: JKP2; P3: JKP3; P4: JKP4; P5: JKP5; and C: control (without treatment) were used as a comparison

Fusarium fungi cause the disease; the infection during the germination phase causes damping off or even death. Infection on grown plants causes the plant to wither and rot in the root, crown root, and base of the stem (Widyastuti et al. 2013). This disease is transmitted through water, agricultural tools, and soil. The pathogen can survive without a plant by forming chlamydospores (resistant structures) and mycelium in the ground (Smail et al. 2017). The fungi produce microconidia, macroconidia, and chlamydospores.

Molecular identification

The molecular identification of the two *Fusarium* species was determined based on the BLAST analysis of JKP2 and JKP3 sequences against the NCBI database (Figure 6). The BLAST result revealed high similarities (99-100%) between the tested sequence and the sequence stored in the database. Based on the most similar result, the JKP2 and JKP3 isolates are identified as *F. oxysporum.*

The phylogenetic analysis confirmed that the two tested isolates are *F. oxysporum* species, as mentioned above. The phylogenetic analysis for the gene fragment coding 28S rDNA using the ITS1 and ITS4 primer pair produces consistent results (Figure 6). The phylogenetic tree formed 2 well-supported clades. Clade 1 includes 9 isolates (ON945579, MG661728, KM268664, HM211241, KJ439169, JKP2, JKP3, OK093403, and EU839375), noted in NCBI as *F. oxysporum*. Clade 2 involved four isolates (PP859259, MH745135, KY922957, MK673882) and *F. oxysporum* NCBI note.

The seed germination in the field is a vulnerable phase of soybean growth where the seeds and seedlings are exposed to pathogenic fungi from the ground, especially in high humidity and low-temperature conditions. Zhang et al. (2024) have produced a list of seven *Fusarium* species related to soybean root rot. In this study, we tested the pathogenicity of five isolates formerly obtained from plants with withering disease in soybean farms. *Fusarium* fungi pathogenicity test should be followed by species identification because many of this genus's members may be responsible for the same symptoms. Accurate fungal pathogen identification often requires a polyphasic approach based on morphology, ecology, and molecular methods (Bhunjun et al. 2021; Arifah et al. 2023).

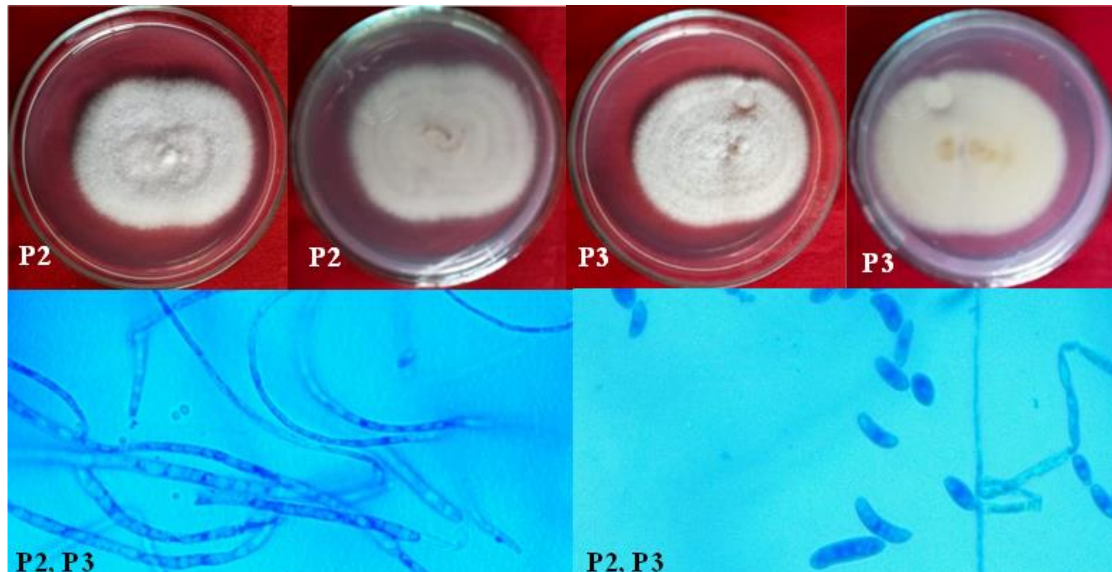

Figure 5. Pure culture and morphology of fungal isolates: P2: JKP2; and P3: JKP3

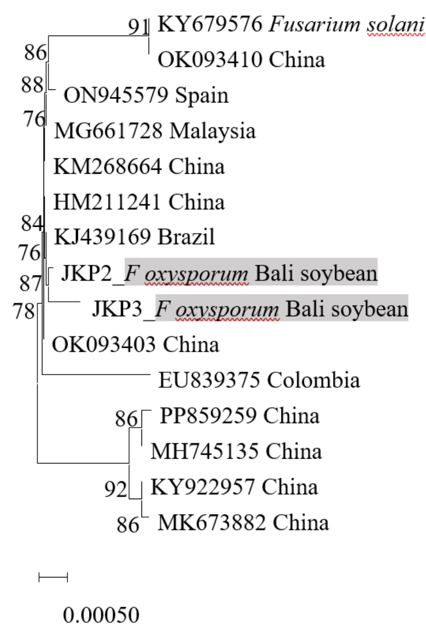

Figure 6. Phylogenetic tree based on the gen 28S rDNA encoder using primer pairs ITS1 and ITS4 Bali isolates. *Fusarium solani* was used as outgroups. Isolates marked with gray highlight are Bali isolates. Bootstraps values greater than 70% based on 1000 replicates are shown on tree branches

Aligning one or two genes has been the standard method to confirm species in the *Fusarium* genus (Summerell 2019). O'Donnell et al. (2022) have recommended region sequencing, which is universally informative in the 28S rDNA coding gene in *Fusarium*. In our study, the 28S rDNA coding gene sequence proved to be sufficient for the unambiguous assignment of the 2 isolates into one *Fusarium* species. Many studies nowadays have been using the same two regions to identify *Fusarium* species (Naeem et al. 2019; Chang et al. 2020a, 2020b; Ma et al. 2021; Félix-Gastélum et al. 2022; James et al. 2022; Xu et al. 2022; Nuangmek et al. 2023). Our study has confirmed that sequencing is important in differentiating *Fusarium* species.

The initial analysis of morphological characteristics such as culture appearance, growth rate, and macroconidia measurements does not show a significant difference between JKP2 and JKP3. The sequencing revealed that JKP2 and JKP3 isolates are the same species, *F. oxysporum*. This is the first report of *F. oxysporum* isolated from soybeans in Bali. The two isolates in this study are included as *F. oxysporum*. All of the isolates appear to be pathogenic on soybean seeds, even though the two *F. oxysporum* isolates (JKP2 and JKP3) clearly are the most aggressive towards soybean of the Anjasmoro cultivar compared to the other isolates. Many reports of *Fusarium* isolate pathogenicity are based on soilless experiments where the germinating soybean seeds were nurtured in a petri dish or paper towel. The results are often similar to our results: all isolates tested are pathogenic but different in aggressiveness (Bonacci and Barros 2019; Chang et al. 2020b). The condition of the pathogenicity test (high humidity and soilless environment) can promote disease development; hence, the result may be too high (Ekwomadu and Mwanza 2023).

Fusarium oxysporum is a ubiquitous species often isolated from plants, including soybeans (Parikh et al. 2018). This species is generally characterized by high genetic diversity, which also affects the aggressiveness. Cultivar in disease severity in disease caused by various *F. oxysporum* isolates is often reported. Olszak-Przybyś et al. (2023) compared the aggressiveness of several isolates from this species, which were obtained from soybean roots in Iowa (AS). All isolates showed harmful effects on the growth of the test soybeans, but only one caused statistically significant root rot compared to the uninoculated control.

In another study, Ellis et al. (2014) characterized more than 100 *F. oxysporum* obtained from soybean roots and seedlings in the US and proved the genetic diversity. Based on laboratory pathogenicity tests, they also categorized the isolates as very aggressive, moderately aggressive, and weak. The most aggressive isolate caused withering, damping off, and clear root rot in soybean seedlings.

The pathogenicity of *Fusarium* spp. is determined by several factors, including the genes responsible for signal transduction, detoxification of antifungal compounds produced by the plants, metabolic enzymes, and cell wall-lysing enzymes (Rauwane et al. 2020). The gene responsible for *Fusarium* pathogenicity, located in an accessory chromosome, can be horizontally transferred from one strain to another (Li et al. 2020). Many *Fusarium* species can produce mycotoxins, such as trichothecene, which can be toxic to plants (Perincherry et al. 2019). The proof that this compound contributes to fungal pathogenicity has been stated, such as by Xu et al. (2023). They found a significant positive correlation between the deoxynivalenol concentration (a trichothecene) and disease index noted for adlay millet inoculated with *F. culmorum* isolate. Our isolates, an *F. oxysporum*, can be used as a subject in similar research in the future. In our pathogenicity test, we found that the inoculated Anjasmoro variant seeds showed very high incidence and disease severity, and the disease area was also extensive. The information that the Anjasmoro variant is vulnerable to *Fusarium* infection may be useful for planning future pathogenicity tests on fungal isolates representing this genus.

In conclusion, five isolates causing withering of soybeans were isolated from soybeans. Through our pathogenicity test, we discovered that only two isolates, JKP2 and JKP3, showed an unprecedented level of aggressiveness, peaking at 98% and causing the highest incidence and most severe disease severity. These two isolates, which were characterized morphologically and molecularly, were found to be members of the *Fusarium* spp. The novelty of our findings was further confirmed by phylogenetic analysis, which identified them as *F. oxysporum.*

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