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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF THE PATHOGENIC FUNGI ISOLATED FROM PURPLE EGGPLANT ORIGINATING FROM BALI, INDONESIA

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SUMMARY

Eggplant (*Solanum melongena* L.) is an important vegetable that is widespread in tropical and subtropical regions. Given that it is a rich source of antioxidants, fibers, minerals, and vitamins, the eggplant is among the top five vegetables in the Asian and Mediterranean regions. The genus *Fusarium* is one of the fungal pathogens that can cause considerable injury to crop plants and grain crops, as well as diseases in humans and animals. The pathogenicity test and molecular analysis for the identification of the casual fungi of wilt disease of purple eggplant were carried out in 2020 at the Mycology Laboratory, Department of Biology, Udayana University, Bali, Indonesia, and Biology Research Center, LIPI, Bogor, Indonesia. The isolated fungi were identified on the basis of macroscopic, microscopic, and molecular characters. The Koch's postulate test also verified the identity of the disease-causing fungal pathogen. Observations based on morphological characters confirmed the genus *Fusarium*. The macroscopic characters of *Fusarium* included creamy white colony mycelia and rapid growth (3 cm in 3 days). Microscopic characters included sickle-shaped macroconidia (27–35 µm long) and oval microconidia (18 µm long). Macroconidia were more abundant than microconidia. The hyphae lacked chlamydospores. On the basis of molecular characters, the species was identified as *Fusarium falciforme* strain DTO 422-HB. These findings are important for managing and controlling wilt disease and enhancing productivity in eggplant.

Keywords: Fungi, *Fusarium falciforme,* macroconidia, microconidia, chlamydospores, wilt disease, *Solanum melongena* L.

Key findings: This study confirmed that the fungal isolate that was identified on the basis of morphological and molecular characters was *F. falciforme*, a causal pathogen of the wilt disease of purple eggplant. The findings of this work are important for managing and controlling wilt disease and enhancing productivity in eggplant in Bali, Indonesia.

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INTRODUCTION

Eggplant (*Solanum melongena* L.) is an important vegetable and ranks first among the 10 types of vegetables; it is also a great source of antioxidants (Sohani *et al*. 2019). The National Diabetes Education Programme, led by both the National Institutes of Health, USA, and the America Diabetes Association (ADA), recommend eggplant as component of the diet for individuals with type 2 diabetes (Caruso *et al*., 2017).

Eggplant contains secondary metabolites, which are natural bioactive compounds and are crucial for good health. Several researchers have examined the chemical composition of eggplant and have extracted nasucin, an anthocyanin that has strong antioxidant activity (Hosseini *et al*., 2016). Ahmed *et al*. (2017) conducted phytochemical tests on purple eggplant fruit extracts and obtained carbohydrates, alkaloids, cardiac glycosides, anthraquinones, flavonoids, terpenoids, steroids, phenols, and saponins. Kowalski *et al*. (2015) identified the chemical composition of three purple eggplant cultivars and found six types of phenolic acids, namely, caffeine, *p*-coumaric acid, ferulic acid, gallic acid, protocatecin, and *p*-hydroxy benzene. Eggplant is useful for the treatment of anti-inflammatory conditions, cardiac debility, neuralgias, nose ulcers, cholera, bronchitis, and asthma (Das and Barua, 2013). Eggplant fruits help lower blood cholesterol levels, act as an antidote to poisonous mushrooms, and exert a hypotensive effect (Diab *et al*., 2011). Eggplant contains mostly water, some protein, fiber, carbohydrates, minerals, vitamins, and low amounts of fats (Satyaprakash *et al*., 2020).

Eggplant, however, is easily infected by disease-causing microbes, leading to reductions in its productivity. Some fungi that cause diseases of eggplant has been reported by researchers in several countries, i.e., *Fusarium oxysporum* f. sp. melongenae has been reported by researchers in Iran (Adhikary *et al*., 2017) and Bangladesh (Safikhani *et al*., 2013). *Mucor* sp. and *Rhizoctonia solani* causes disease in Nsukka, Enugu State, Nigeria (Gambari *et al*., 2013). The fungus *Diaporthe vexans* causes fruit rot and leaf blight diseases in India (Mahadevakumar and Janardhana, 2016).

In Indonesia, eggplant is also a very popular vegetable crop. However, eggplant crops are easily infected by some diseases, and eggplant productivity tends to decrease annually. Diseases, such as anthracnose caused by *Phomopsis vexans*, leaf spot, and *Fusarium* wilt reduce eggplant fruit productivity (Nahar *et al*., 2019; Sujudi *et al*., 2020). Moreover, fruit rot disease caused by the fungus *P. vexans* and *Colletotricum melongena* adversely affect eggplant productivity in Rancabungur and Kemang Districts, Bogor Regency, Indonesia (Hakikah, 2013). Meanwhile, leaf spot caused by the fungus *Alternaria s*p. and fruit rot caused by the fungus *Colletotrichum* have been observed in eggplant in the Baturaden area, Banyumas Regency, Indonesia (Sucianto and Abbas, 2019).

Preliminary research results obtained at several locations in Bali indicate that eggplant is attacked by fungal diseases. However, studies on the pathogenic fungi that causes diseases of eggplant are lacking. In the field research on purple eggplant conducted in September 2019 at Subak Sembung, Peguyangan Village, North Denpasar District, Bali, Indonesia, eggplant diseases were identified, and more than 270 plants were found to be infected with wilt disease. Other cases of eggplant wilt disease were also found in Subak Sindu Jiwa, Sayan Village, Ubud, Gianyar; Belok Sidan Village, Plaga, Badung; Perean Village, Baturiti; Tabanan, Indonesia.

Field observations and analysis revealed that the cause of the wilt disease of eggplant is a pathogenic fungus considering that the plants infected with fungus presented infected roots that were odorless and not slimy after water immersion and lacked the milky white mucus appearance of bacteria. Past studies have observed that wilt disease

causes sunken but not rotten black spots on eggplant fruits (Soesanto *et al*., 2013; Mihovilovich *et al*., 2017). The problem addressed in this research was the identity of the pathogenic fungi that caused wilt disease in purple eggplant from Bali. The objective of this study was to identify the pathogen causing fungal eggplant disease in Bali by using morphological and molecular characters. The finding of this study would provide scientific evidence that is important for further developing biopesticides and strategies for eggplant disease eradication.

MATERIALS AND METHODS

The morphological identification of fungi was conducted from January 2020 to September 2020 at the Mycology Laboratory, Department of Biology, Udayana University, Bali, Indonesia. The molecular analysis was carried out in November 2020 at the Biology Research Center, LIPI, Bogor, Indonesia

Research material

The research material comprising purple eggplant plants was obtained from Subak Sembung, Peguyangan Village, North Denpasar, Bali, Indonesia. The pathogenic fungi were isolated from disease-stricken eggplant plants. The chemicals used in this study were 70% ethanol, bayclin (5.25% NaOCl), and potato dextrose agar (PDA).

Research instruments

The instruments used in this research were a laminar flow cabinet, aluminum foil, petri dishes, microscope (Nikon), one needle, Bunsen lamp, blender, balance, knife, autoclave, a set of glass tools, and a polymerase chain reaction apparatus (Fisher Scientific Thermo Scientific "Arctic" Thermal Cycler) with ABI 3730 xl sequencer.

Procedures

Isolation of pathogenic fungi from purple eggplant

Pathogenic fungi were isolated by cutting 0.5 cm × 0.5 cm samples from the pathogen-infected parts (the roots, bark, leaves, and fruits) of the eggplant plants. Sample pieces (four pieces) were sterilized with 5.25% NaOCl for 2 min and washed three times with sterile water and dried. The samples were placed in a petri dish that already contained PDA media (Figure 1). All the samples were incubated at room temperature (25 °C to 27 °C) for 5 days. Mycelia that grew during an incubation period of 3 days was then transferred to new PDA media to obtain pure fungal cultures (Bechem and Afanga, 2017).

The isolates of the pure fungi were then tested in accordance with Koch's postulates, i.e., the roots of 3-week-old eggplant seedlings were cleaned free of dirt, cut at 0.5 cm from the root tip, dipped in a suspension of fungal spores for 5 h, and then planted in polybags. As a control, eggplant roots were cut at the ends, immersed in water, and then planted in polybags.

Morphological symptoms were observed and compared with disease symptoms in the field. The pathogens were then reisolated from the infected plants. The obtained pure cultures of fungal pathogens were inoculated into healthy eggplant plants. The same inoculation procedure was performed three times to obtain similar symptoms. The fungal isolate was the cause of the wilt disease of eggplant.

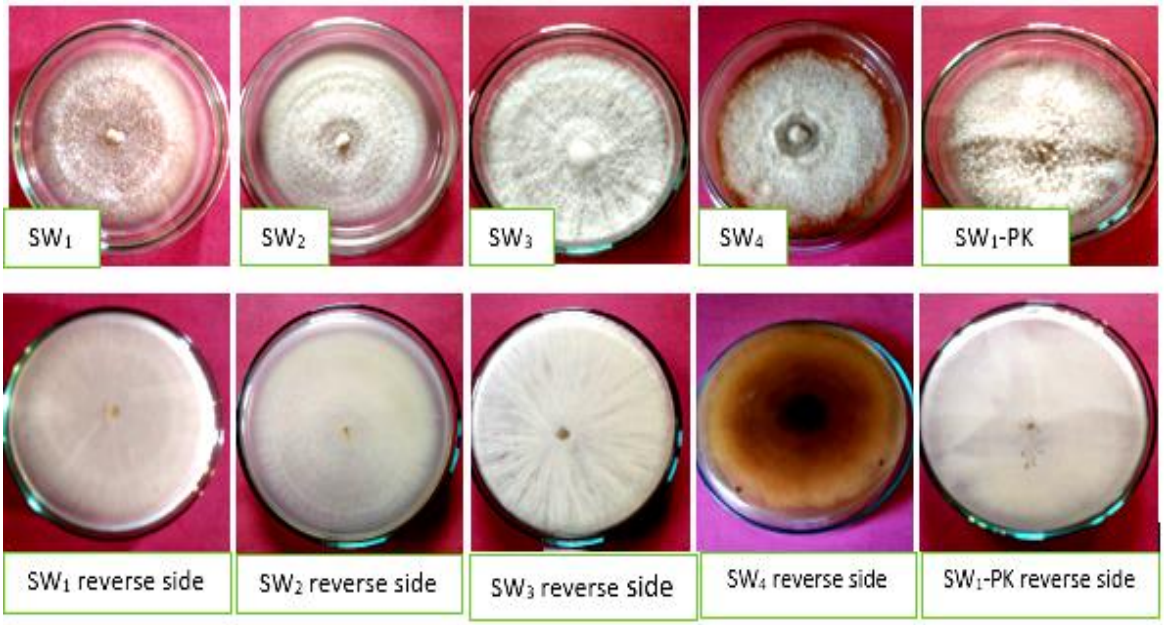

Figure 1. Five isolates of fungi (SW1, SW2, SW3, SW4, SW5, and SW1-PK) on PDA Media.

Characterization of the pathogenic fungi of purple eggplant plants

Morphological characterization

The morphological identification of pathogenic fungi was carried out on the basis of macroscopic and microscopic characters. Macroscopic characters included the color of the fungal colony, the reverse color of the colony, the surface shape of the colony, and the growth rate of the fungus. Microscopic observations on the shape of the hyphae, spore shape, and spore size were performed under a microscope in reference to a fungal identification book.

Molecular characterization

The molecular identification of pathogenic fungi in purple eggplant was based on the partial genetic analysis of the internal transcribed spacer (ITS) locus of ribosomal fungal DNA. DNA isolation was carried out by isolating fungi in potato dextrose broth medium. The isolated fungi were then incubated for 72 h. The fungal mycelia biomass was then taken for DNA extraction. Fungal DNA extraction was carried out by using pure PHYTO nucleon reagent (Amersham LIFE SCIENCE). The PCR amplification of the ITS used the following ITS primers: 4:5'–TCC GCT TAT TGA TAT GC–3' and 5:5'–GGA AGT AAA AGT CGT AAC AAG G–3' (Smith *et al*., 2017).

The PCR results were purified by using the PEG precipitation method (Lili *et al*., 2013) and then subjected to sequencing. The sequencing results were purified again via the ethanol purification method. Nitrogen base sequence readings were analyzed by using an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The raw data from the sequencing were then trimmed and assembled by using the Bio-edit program (http://www.ncbi.nlm.nih.gov/BLAST/). Sequence data were assembled further in BLAST with genomes and were registered at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) to determine the species with great homology and adjoining molecularly.

RESULTS AND DISCUSSION

Isolation of pathogenic fungi from purple eggplant

The pathogenic fungi were collected from wilt-infected purple eggplant plants in Subak Sembung, Peguyangan Village, North of Denpasar, Bali, Indonesia. The pathogenic fungi that were isolated from the roots, rhizosphere, fruit, leaves, and bark were designated as SW_1, SW_2, SW_3, SW_4, and SW_5, respectively, in accordance with the observations. The results for the purification of the fungal isolates were then collected. Reisolation was performed on the third day. SW1, SW2, SW3, and SW4 isolates formed colonies with white mycelia, whereas the mycelial isolates from the SW5 colony were black. The color and shape of the mycelium were considered as macroscopic characters. In accordance with the morphological macroscopic and microscopic characters, the isolates SW1, SW2, SW3, and SW4 were identified as *Fusarium* spp, and the SW1 fungal isolate was identified as a *Fusarium* fungus (SW1-PK) on the basis of Koch's postulates.

The results of purification of fungal isolates through reisolation after inoculation on the third day showed that the mycelia of SW_1, SW_2, SW_3, SW_4, and SW_1-PK were white, whereas those of SW_5were black. The Koch's postulate test was carried out on SW_1, SW_2, SW_3, SW_4, and SW_5 and showed that the symptoms of wilt disease in the samples were similar to those in the field (Figure 2). Ten purple eggplant plant samples were subjected to the Koch's postulate test. Four plants exhibited abnormal growth and stunting, and two samples had wilt disease.

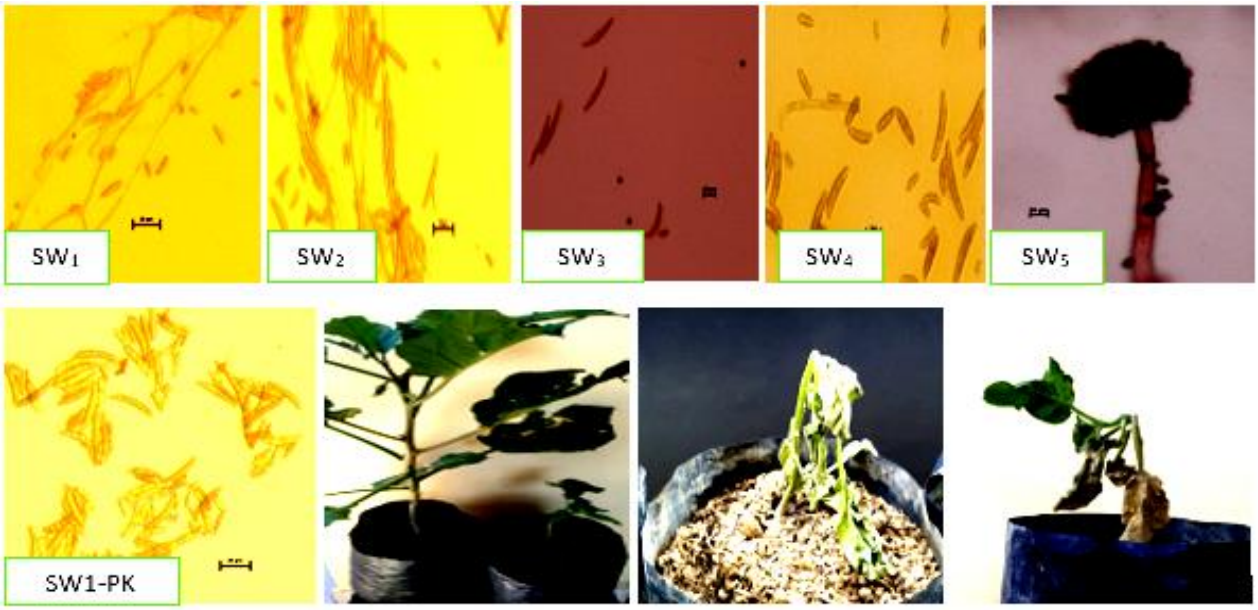

Figure 2. Microscopic characters of the isolates of fungi (SW1, SW2, SW3, SW4, SW5, and SW1-PK) and Koch's postulate test of pathogenic fungi in purple eggplant.

Characteristics of pathogenic fungi

Morphological characteristics

SW_1, SW_2, SW_3, SW_4, and SW_1-PK exhibited white mycelia. The samples had cream reverse colony colors. However, the reverse colony color of SW_4 was brown. The samples had serrated fungal shapes, flat surface shapes, colony growth pattern, and very fast colony growth (3.03 cm to 3.50 cm in 3 days) on PDA media. On the basis of the identification results, the isolate was identified as *Fusarium* spp. The SW_5 isolate was thought to be *Aspergillus niger* given its macroscopic characteristics of black color, hooded fungal shape, and spreading growth. Silva *et al*. (2011) stated that *A. niger* has black-to-dark brown mycelia, colorless-to-light yellow reverse colonies, and finely wrinkled/wrinkled and globular/ellipsoidal conidia. According to Hussein *et al*. (2012), the colonies of *F. oxysporum* inoculated on PDA media grew rapidly with diameters of 3.2 cm to 4.5 cm in 4 days.

Aerial mycelia were sparse or floccose; velvety, whitish, or peach usually with a purple tinge; intense near the medium surface; and purple in reverse. Hafizi *et al*. (2013) revealed that the fungus *Fusarium soloni* inoculated on PDA media is very fast-growing with a colony diameter of 3.3 cm to 3.5 cm in 4 days. Its aerial mycelia are sparse or dense and floccose, sometimes leathery, grayish-white, and cream to buff. Duarte *et al*. (2019) observed that the fungus *F. falciforme* infects *Phaseolus vulgaris* beans in Cuba and forms white mycelia and creamy reverse colonies at the growth rate of 4.6 mm per hour at 25 °C ± 2 °C when inoculated on PDA media. Sousa *et al*. (2020) found that *F. falciforme* from *Phaseolus lunatus* nuts from Brazil form white and yellowish colonies and cream reverse color after 7 days of inoculation on PDA media. Gutierrez *et al*. (2019a) reported that the fungus *F. falciforme*, which causes root and stem rot in papaya (*Carica papaya*) in Mexico, exhibits white to creamy mycelia when inoculated on PDA media.

Based on the microscopic characters, the isolate SW_1 exhibited crescent-shaped macroconidia with 4–5 septa that were 28–30 µm long, oval microconidia with 1–2 septa and lengths of 15–18 µm, and septate hyphae. The isolate SW_2 was recorded with crescent-shaped macroconidia with 4–5 septa that were 35–42 µm long, microconidia with 1–2 oval-septa and lengths of 11–21 µm, and septated hyphae. The isolate SW_3 was reported with 4–5 septa crescent-shaped macroconidia and lengths of 25–28 µm, oval microconidia, and septa that were 2.8 µm long. The isolate SW_4 showed crescent-shaped macroconidia with 4–5 septa with lengths of 38–45 µm and oval microconidia with 1–2 septa and lengths of 20–25 µm. Isolate SW_1-PK had macroconidia with 4–5 crescent-shaped septa that were 27–35 µm long and oval microconidia with 1–2 septa that were 14–18 µm long. The macroconidia were more abundant than microconidia, and chlamydospores were not formed.

The results of microscopic characteristics indicated that the isolates SW1, SW2, SW3, SW4, and SW1-PK were *Fusarium* spp. Hussein *et al*. (2012) reported that the fungus *F. oxysporum* exhibits crescent-shaped macroconidia with 3–5 septae that are 20, 27–40, 50 µm long and 0–2 oval microconidia septa that are 7.50–16.25 µm long. Hafizi *et al*. (2013) found that *Fusarium solani* has macroconidia with a length of 27–46.2 µm and 0–1 oval-shaped microconidia with a length of 8–16 µm. The macroconidia are crescent-shaped and contained 3–5 septa. Duarte *et al*. (2019) reported that the fungus *F. falciforme* shows crescent-shaped macroconidia with 3–5 septa and oval-shaped microconidia with 0–1 septa. The chlamydospores are singly formed, in clusters, chains, and terminals in pairs. Sousa *et al*. (2020) revealed that the fungus *F. falciforme* exhibits crescent-shaped 3–4-sided macroconidia that are 17.7–32.8 µm in length and 0–2 oval-shaped microconidia that were 9–18.6 µm in length. Gutierrez *et al*. (2019b), in the identification of *F. falciforme*, observed three-sided crescent-shaped macroconidia

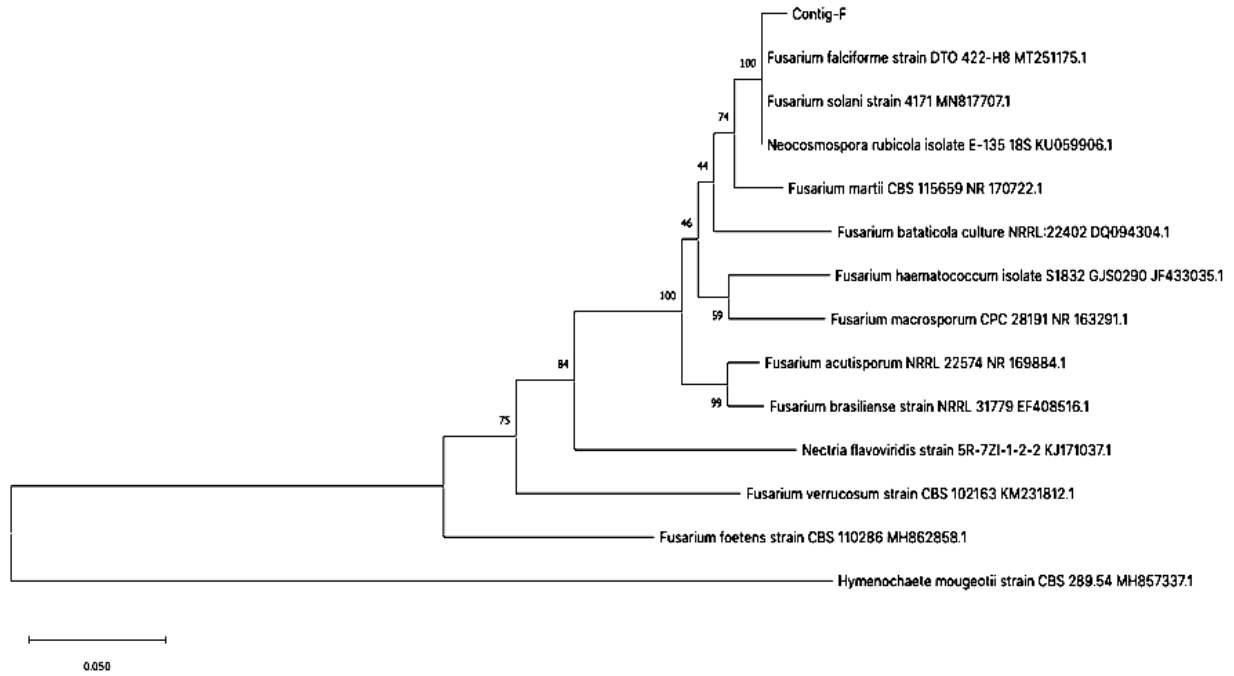

Figure 3. Phylogenetic tree of *F. falciforme* obtained via neighbor-joining method and taxonomic evolutionary relationships.

that are 23.7−51.2 µm in length and possessing 0–2 oval-shaped microconidia with a length of 4.8–11.2 µm. Chlamydospores are not found.

Molecular characteristics

Molecular characteristics were revealed by the PCR amplification results of the isolate SW_1-PK fungus with the ITS primers 4:5'–TCC GCT TAT TGA TAT GC–3' and 5:5'–GGA AGT AAA AGT CGT AAC AAG G–3'. The sequencing results that were assembled then BLASTed with genomes that have been registered in the NCBI showed that the isolate SW_1-PK had 99.29% similarities with *F. falciforme* strain DTO 422-HB and *F. falciforme* strain DTO 421-G2. The results of the BLAST analysis were supported by phylogenetic results with a bootstrap value percentage of 100 (Figure 3). Siahpoush and Darvishnia (2018) have discovered new *Fusarium* species from Poaceae in Western Iran: *F. falciforme, Fusarium concentricum*, and *Fusarium torulosum*. By using PCR, Gutierrez *et al*. (2019a) identified the *Fusarium* fungus as the cause of footrot and wilt diseases of tomato plants in Sinaloa, Mexico, and found that *F. falciforme* had a homology of 99.9% and *F. oxysporum* had a homology of 99.9% to 100%. Moreover, Gutierrez *et al*. (2019b) stated that *F. falciforme* is more aggressive in infecting tomato plants than *F. oxysporum.*

CONCLUSIONS

The pathogenic fungus showed the following morphological characteristics: white mycelia, rapid colony growth, and cream reverse colony color. It presented the following microscopic characters: 4–5 sickle-shaped macroconidia and 1–2 oval-shaped microconidia. Molecular characters were obtained through the alignment of the base series and the phylogenetic analysis of genetic relationships. The fungus *F. falciforme* was identified as the major causal agent of the wilt disease of purple eggplant plants from Bali, Indonesia. The present findings could help

researchers and growers manage and control wilt disease and enhance productivity in eggplant in the future.

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