MYCOBIOLOGY
2023, VOL. 51, NO. 3, 186–194
https://doi.org/10.1080/12298093.2023.2213911

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Characterization of Three *Fusarium* spp. Causing Wilt Disease of *Cannabis sativa* L. in Korea

Young Mo Koo[a], S. M. Ahsan[a] and Hyong Woo Choi[a,b]

[a]Department of Plant Medicals, Andong National University, Andong, Korea; [b]Institute of Cannabis Biotechnology, Andong National University, Andong, Korea

ABSTRACT

In July 2021, wilting symptoms were observed in adult and seedling hemp (*Cannabis sativa* L. cv. Cherry Blossom) plants grown in a greenhouse. As the disease progressed, yellowing and wilting symptoms on the leaves developed, resulting in whole plant death. In seedling plants, typical damping-off symptoms were observed. To identify the pathogen, the roots of diseased plants were sampled, surface sterilized, and cultured on potato dextrose agar (PDA) media. From the culture, 4 different fungal isolates were recovered and purely cultured. Each fungal isolate showed distinct growth shapes and color development on malt extract agar, oatmeal agar, sabouraud dextrose agar, and PDA media. Microscopic observation and molecular identification using ribosomal DNA internal transcribed spacer sequencing identified them as 3 *Fusarium* spp. and 1 *Thielaviopsis paradoxa*. Additional sequencing of elongation factor 1-alpha and β-tubulin regions of 3 *Fusarium* spp. revealed that 2 of them are *Fusarium solani*, and the other one is *Fusarium proliferatum*. To examine which isolate can act as a causal agent of wilt disease of hemp, each isolate was tested for their pathogenicity. In the pathogenicity test, *F. solani* AMCF1 and AMCF2, and *F. proliferatum* AMCF3, but not *T. paradoxa* AMCF4, were able to cause wilting disease in hemp seedlings. Therefore, we report that *F. solani* AMCF1 and AMCF2, and *F. proliferatum* AMCF3 as causal agents of Fusarium wilt of hemp plants. To our knowledge, this is the first report of the wilt disease of *C. sativa* L. caused by *Fusarium* spp. in Korea.

ARTICLE HISTORY
Received 2 February 2023
Revised 26 March 2023
Accepted 10 May 2023

KEYWORDS
Cannabis sativa L; Hemp; *Fusarium* wilt

1. Introduction

Historically, hemp plants (*Cannabis sativa* L.) have long been cultivated for versatile use, such as food, textile, and medicine [1]. Recently, hemp plants getting more and more attentions due to their (i) high carbon-sequestering properties, (ii) high biomass production properties, and (iii) various end-use [2–4]. More importantly, hemp is a great source of cannabinoids, such as cannabidiol (CBD) and tetrahydrocannabinol (THC), which seem to be able to relieve or treat multiple human diseases. Therefore, the area cultivated with hemp is drastically increasing worldwide, including Korea.

Like other crop plants, the production of hemp plants can be suffered from various microbial pathogens. In hemp plants, different fungal, oomycete, bacterial, and viral pathogens are known to cause specific diseases in different organs [5]. Different fungal pathogens, such as *Alternaria alternate*, *Botrytis cinerea*, *Botrytis pseudocinerea*, *Fusarium equiseti*, and *Fusarium graminearum* are known to cause bud rots of hemp plants. Of these pathogens, *Fusarium* spp. are among the most destructive pathogens of *C. sativa*. This is especially evident during the vegetative growth phases as infection results in decreased plant quality and total plant loss [6,7]. *Fusarium* spp. are also responsible for reducing crop value by producing mycotoxins, such as deoxynivalenol, zearalenone, and fumonisin B [7]. In hemp plants, *Fusarium* diseases are known to induce vascular wilts, however, some species can cause seedling damping-off, and rot of the crown, lower stem, root, and seed, as well as head and grain blights.

For *Fusarium*, the concept of "species complex" was proposed from a phylogenetic context and brings together groups of species that share many important practical characteristics, such as toxin production and other phenotypic features [8,9]. Recently, two taxonomic structures for the genus have been proposed. The first focuses on micromorphology and morphology of asexual spores in addition to molecular data [10]. The second solely relies on a molecular data-driven taxonomy [11]. Among different *Fusarium* spp., *Fusarium solani* and

CONTACT Hyong Woo Choi hwchoi@anu.ac.kr

Fusarium oxysporum are the major species involved in root and stem rots. Other *Fusarium* species, such as *Fusarium avenaceum, Fusarium culmorum, Fusarium graminearum,* and *Fusarium fujikuroi*, reportedly induce stalk and root rots [7]. Recently, 16 species in the genus *Fusarium* have been evidenced as associated with different diseases of hemp plants [7,11]. In hemp seedling plants, infection by Fusarium wilt pathogens induces a stunting phenotype, and eventual wilt and die. In older plants, mature leaves become chlorotic, often only on one side of the plant, and eventually, the whole plant collapses.

In this study, we observed wilting symptoms of hemp (*C. sativa* L. cv. Cherry Blossom) plants grown in a greenhouse. To identify the causal agent, pathogen isolation and identification have been performed. We have isolated four distinct fungal isolates and identified them as *F. solani* AMCF1 and AMCF2, *Fusarium proliferatum* AMCF3, and *Thielaviopsis paradoxa* AMCF4 based on morphological and molecular studies. Among these, *F. solani* AMCF1 and AMCF2, and *F. proliferatum* AMCF3 were able to induce wilting (or damping-off) symptoms in hemp seedlings, whereas *T. paradoxa* AMCF4 was not. Thus we report that three pathogenic *Fusarium* strains and one nonpathogenic fungal strain from one newly introduced variety of *C. sativa*. To our knowledge, this is the first report of a *Fusarium* wilt of hemp plants in Korea.

2. Materials and methods

2.1. Plant growth condition

Hemp plants (*C. sativa* L. cv. Cherry blossom; Blue Forest Farms, USA) were grown in coco peat as a medium. Hemp plants were grown at vegetative growth condition for two months at 25 °C, relative humidity (RH) less than 60% and light intensity of ~300 µmol/m^2/s for 18 h (6 h dark). After completion of the vegetative growth period, hemp plants were grown at reproductive growth conditions for one month at ~25 °C, less than 60% RH, and light intensity of ~300 µmol/m^2/s for 8 h (16 h dark) in order to promote flowering.

2.2. Fungal isolation

During the growing the hemp plants in green house, some of them showed wilting and damping of symptoms. Symptomatic roots or stems were collected (10 mm long), surface sterilized by soaking in 1% NaOCl for 1 min, 70% ethanol for 1 min, and then rinsed with sterile water three times [12]. Surface sterilized samples were placed on potato dextrose agar media (PDA; MBcell, Seoul, Korea), and incubated at 25 °C for three to five days. Each fungal colony was transferred to fresh PDA media and stored in 20% glycerol at −80 °C.

2.3. Morphological observation

For morphological observation, each fungal isolate was grown on PDA, oat meal agar (OA; MBcell), Malt extract agar (MEA; MBcell) and Sabouraud dextrose agar (SDA; MBcell) media at 25 °C. Representative colony shape and color development pictures were taken at seven days after inoculation on each media. To observe the spore, each fungal isolate was grown on PDA or carboxymethyl cellulose agar (CMCA; 0.5 g CMC, 1 g $MgSO_4.7H_2O$, 1 g NH_4NO_3, 1 g KH_2PO_4, 1 g yeast powder and 15 g agar/L) media for seven days. The structure of spores was observed with light microscopy at 400x (Olympus JP/BX50, Olympus, Tokyo, Japan). The average dimensions of spores were recorded from 100 spores per isolate. ImageJ was used to measure the length and width of spores [13].

2.4. Molecular identification and phylogenetic analyses

Fungal genomic DNA was extracted by using HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) following the manufacturer's protocol. Initially, the internal transcribed spacer (ITS) region of 18S–28S nuclear DNA (rDNA) was amplified using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS2 (5′-TCCTCCGCTTATTGATATGC-3′) [14]. PCR was performed in 30 µl reactions of Solg™ 2X Taq PCR Pre-Mix (SolGent, Daejeon, Korea) containing 20 pmol of each primer and approximately 20 ng of template DNA. PCR amplification was performed as previously described [15]. Briefly, PCR was carried out using thermal cycler (Multigene Gradient, Labnet, Edison, NJ, USA) by following amplification conditions: denaturation at 95 °C for 5 min, 33 cycles of 94 °C for 30 s, 56 °C for 10 s, 72 °C for 40 s, and final extension at 72 °C for 10 min. PCR products were analyzed by gel electrophoresis and purified using PCR purification Kit (BIOFACT, Daejeon, Korea) according to the manufacturer's instructions for sequencing. The resulting sequences of the ITS rDNA of AMCF1, AMCF2, AMCF3 and AMCF4 were analyzed with NCBI's GenBank sequence database (http://www.ncbi.nlm,nih.gov) to identify the closest species relatives and phylogenetic tree was constructed by MEGA-X software using maximum likelihood method [16]. To further identify *Fusarium* spp., additional sequencing of elongation factor 1-alpha (EF) and β-tubulin (TUB) regions

were amplified and sequenced by using the specific primer sets (EFF: 5′-ATGGGTAAGGARGACAAG AC-3′, EFR: 5′-GGARGTACCAGTSATCATGTT-3′, TUBF: 5′-AACATGCGTGAGATTGTAAGT-3′, TUBR: 5′-TCTGGATGTTGTTGGGAATCC-3′,) [11].

2.5. Pathogenicity tests

One-week-old hemp seedlings were inoculated by dipping the root in the spore suspension (10^8 spores/ml) of each fungal isolate for 30 min. Infected plants were maintained at high humidity condition by using the transparent plastic dome. For each fungal isolate, five plantlets were used for infection and disease severity was monitored at seven days post inoculation (dpi). Fungal pathogens were reisolated from symptomatic stems and sequenced for identification to fulfill Koch's postulates.

3. Result

3.1. Pathogen isolation and identification

In 2021, severe wilting and damping off symptoms were observed in a greenhouse and nursery of hemp plants, respectively, which is located in Andong National University (36°32′39.5″N 128°47′58.9″E) (Figure 1). Regardless of enough irrigation, mature plants start to show wilting symptoms and eventually died (Figure 1(A–C)). In addition, hemp seedlings sown coco peat media exhibited severe damping-off symptoms (Figure 1(D)). Over 70% of seedlings developed damping off symptoms and died eventually. As the disease progressed, white-colored mycelial growth was observed on the basal part of the stem, which seems likely caused by fungal infection and proliferation.

To isolate the causal pathogen, root samples were collected from symptomatic plants, surface sterilized, then incubated on PDA media. Fungal growth was

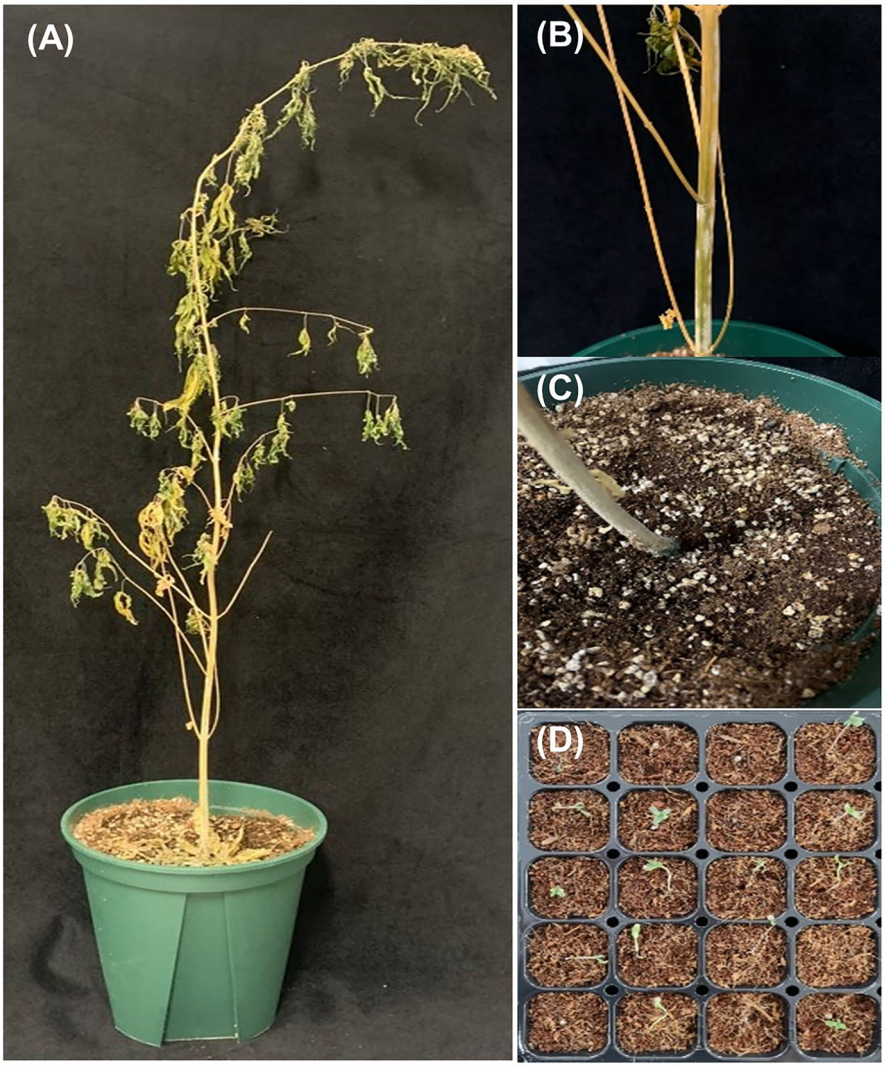

Figure 1. Wilting symptoms of *Cannabis sativa* L. cv. Cherry Blossom (hemp) plants. (A) Wilting symptoms developed on the flowering stage of hemp plants; (B–C) Basal part of the stem of diseased hemp plants. White-colored mycelial growth is observed; (D) Wilting symptoms of hemp seedlings plants.

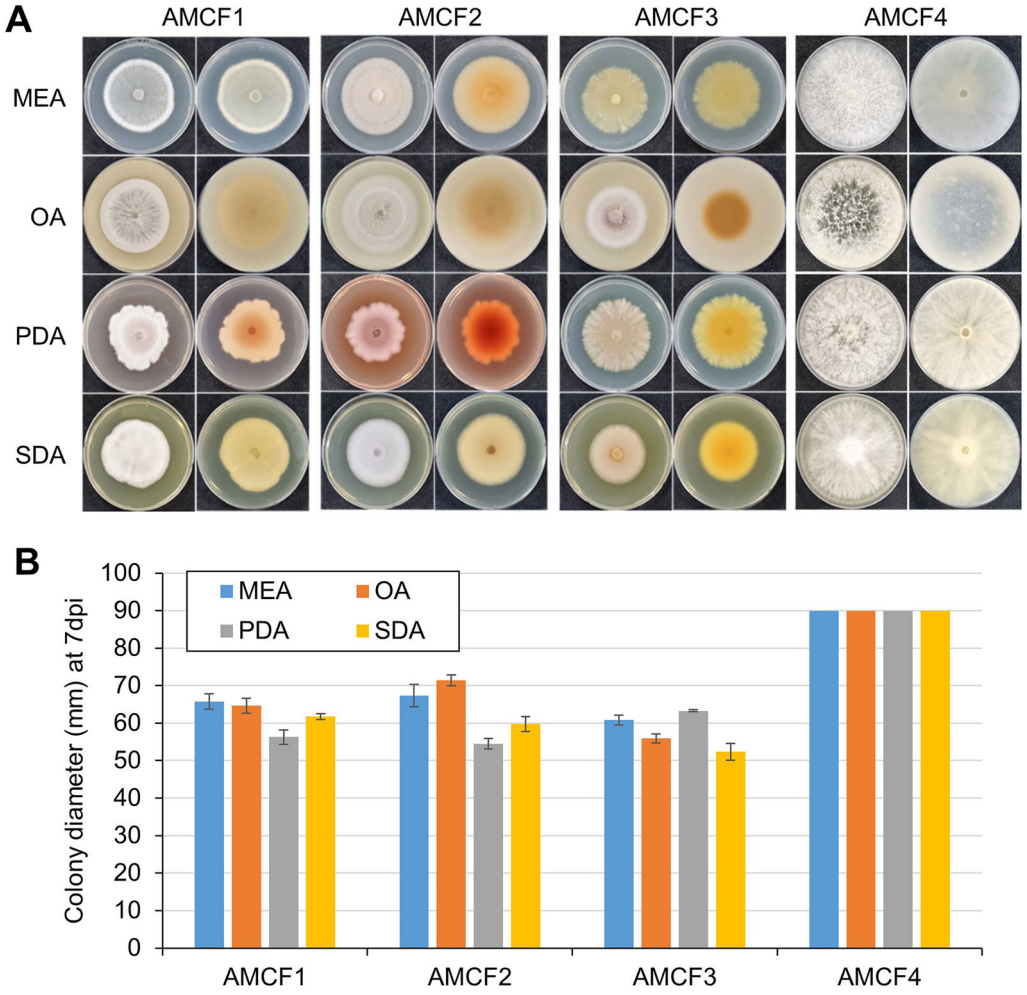

Figure 2. Growth phenotypes of AMCF1, AMCF2, AMCF3, and AMCF4 on different media. (A) Colony morphology of each fungal isolate on malt extract agar (MEA), oatmeal agar (OA), potato dextrose agar (PDA) and sabouraud dextrose agar (SDA) media at 25 °C for seven days. Left and right rows of each figures are upside and downside views of mycelial growth on each media, respectively; (B) Colony diameter of each fungal isolate on different media at seven days post inoculation (dpi).

visually observed as they grew. Four different fungal isolates were collected and purely cultured and named AMCF1, AMCF2, AMCF3, and AMCF4 for further experiments. On all tested MEA, OA, PDA, and SDA media, AMCF4 showed the fastest growth as it filled up a 90 mm-diameter Petri dish at seven-days post incubation (dpi) (Figure 2(A)). Colony morphology of AMCF1 and AMCF2 were somehow similar to each other as they developed circular-shaped colony on MEA, OA, and SDA, but showed irregularly round-shaped marginal growth on PDA. Unlike AMCF1 and AMCF2, the AMCF3 colony showed sharp irregular margins on MEA and PDA, but a circular shape on OA and SDA. Each isolate also developed distinct colors on different media. AMCF1 showed a white-colored colony on MEA, OA, and SDA, but a pale orange color on PDA. AMCF2 colony developed strong purple coloration on PDA media, while AMCF3 showed purple color in OA. Unlike other isolates, AMCF4 did not express coloration on all the tested media. As mentioned above, AMCF4 grow up to 90 mm-diameter in all tested media at 7 dpi (Figure 2(B)). But, other strains, AMCF1, AMCF2, and AMCF 3, showed distinct, but similar growth on all tested media. Among these three isolates, in MEA and SDA media, AMCF1 and AMCF2 grow better than AMCF3; however, in PDA media, AMCF3 grows better than AMCF1 and AMCF2 (Figure 2(B)). Taken together, AMCF1 and AMCF2 showed somehow similar growth pattern in their growth speed and shape, but the growth of AMCF3 and AMCF4 were somehow different from AMCF1 and AMCF2.

On PDA media AMCF1, AMCF2, AMCF3, and AMCF4 produced microconidia (Figure 3(A–H)). Microconidia of AMCF1, AMCF2, and AMCF3 were elongated oval shapes. The size of microconidia of AMCF1, AMCF2, and AMCF3 were 2.3–4.6 × 0.6–0.9 µm, 3.1–3.8 × 0.6–0.9 µm, and 2.6–5.0 × 0.5–1.0 µm, respectively, which seem to be similar with *Fusarium* spp. [17–19]. To observe macroconidia, these fungal isolates were grown on CMCA media for seven days [20]. However, we were only able to observe macroconidia of AMCF2,

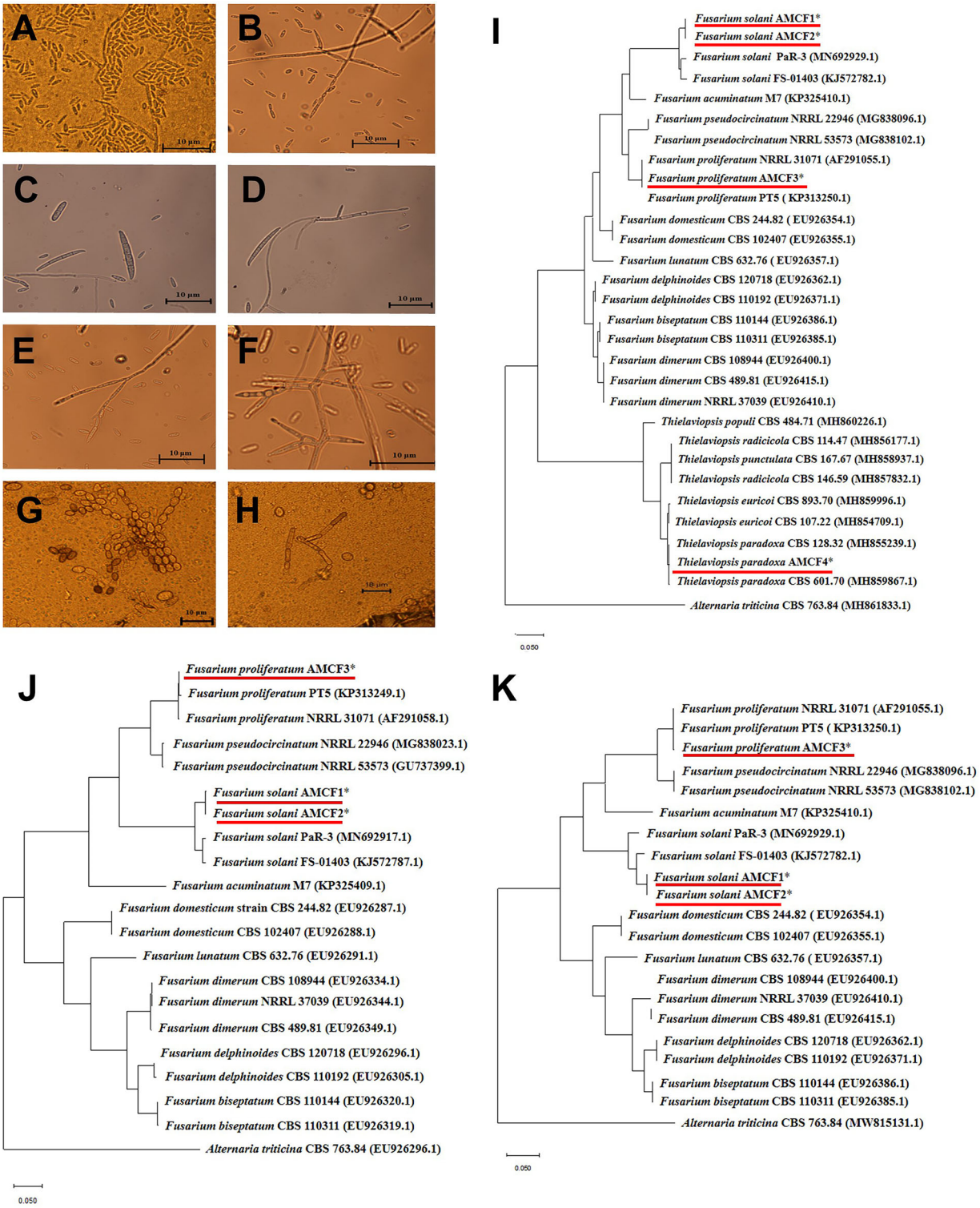

Figure 3. Morphological and molecular identification of AMCF1, AMCF2, AMCF3, and AMCF4. (Microscopic observation of AMCF1 (A and B); AMCF2 (C and D); AMCF3 (E and F); AMCF4 (G and H). Scale bar = 10 µm. Phylogenetic analyses of AMCF1, AMCF2, AMCF3 and AMCF4. The tree was constructed based on internal transcribed spacer (ITS) (I); translation elongation factor 1-alpha (TEF) (J); beta-tubulin (TUB) (K). Phylogenetic tree were generated by maximum likehood method analysis with bootstrap values of 1000. The ITS, TEF and TUB sequences of *Alternaria triticina* strain CBS 763.84 were selected as an outgroup to root the phylogeny. Scale bar indicated a phylogenetic distance of 0.05 nucleotide substitutions per position.

which were slightly curved, hyaline, and pointed end with three septa. The size of the macroconidia of AMCF2 was 11.5–14.6 × 0.7–1.5 µm. AMCF4 produced conidia on PDA media, which was an oval shape, but not an elongated oval shape, and their size was measured as 3.3–4.4 × 1.6–2.2 µm. Overall shape and size of microconidia of AMCF1, AMCF2, and AMCF3 were somehow similar to each other but different from those of AMCF4.

For molecular identification, the ITS region of each isolate was sequenced and analyzed by constructing the phylogenetic tree (Figure 3(I)). From

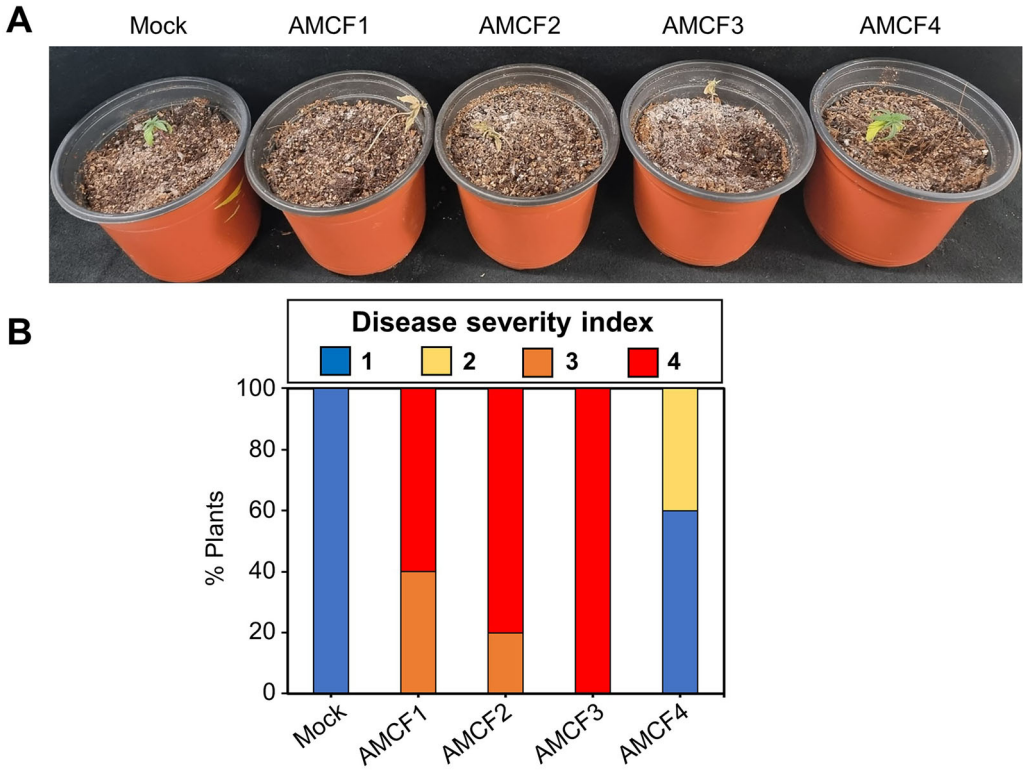

Figure 4. Pathogenicity test of the *Fusarium solani* AMCF1 and AMCF2, *Fusarium proliferatum* AMCF3 and *Thielaviopsis paradoxa* AMCF4 on hemp seedlings. (A) Representative disease symptoms of hemp plants at 7 dpi with indicated isolate of fungi. For mock treatment, the roots were submerged into sterile tap water; (B) Disease severity of hemp plants inoculated with indicated fungal strains. Stacked bar plots indicated the % plants showing indicated disease severity. Disease severity index: 1 = no symptoms (blue); 2 = Discoloration in the lower leaves (yellow); 3 = All the leaves are showing wilting symptoms (brown); 4 = plant death (red).

the analysis, AMCF1 and AMCF2 belong to the *F. solani* species complex, while AMCF3 and AMCF4 were identified as *F. proliferatum* (*F. fujikuroi* species complex) and *T. paradoxa*, respectively. To further identify *Fusarium* species (AMCF1, AMCF2, and AMCF3) additional sequencings of elongation factor 1-alpha (EF) and β-tubulin (TUB) regions were performed and used for phylogenetic analysis (Figure 3(J,K)). Further analysis using EF and Tub revealed that AMCF1 and AMCF2 are indeed *F. solani*, and AMCF4 is a *F. proliferatum*.

3.2. Pathogenicity test of isolated fungi

Four different fungal isolates were further tested for their pathogenicity in hemp seedlings. One-week-old hemp seedlings were inoculated with spore suspensions of each fungal isolate (1 x 10^8 spores/ml). As shown in Figure 4(A), AMCF1, AMCF2, and AMCF3 successfully induced wilting symptoms on hemp seedling plants, while AMCF4 failed to induce disease symptoms (severity index 1-2). In mock-treated plants, all seedlings showed no disease symptoms (severity index 1), while the most of AMCF1-, AMCF2-, and AMCF3-inoculated plants showed severe symptoms (severity index 3) or death (Figure 4(B)). The fungal pathogens were re-isolated from the diseased hemp plants and sequenced for identification. AMCF1, AMCF2, and AMCF3 were successfully isolated and identified from the infected plants, suggesting these *Fusarium* spp. are indeed a causal agent of Fusarium wilt disease of hemp plants.

4. Discussion

Cultivation of hemp plants (*C. sativa* L.) getting more and more increased worldwide for centuries [5]. Hemp plants are the major source of some phytocannabinoids, such as the Δ9-tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), and cannabichromene (CBC), all of which are currently under extensive research. The legalization of hemp plants in different countries, including the USA, Canada, and some EU countries, for medicinal use, has increased the interest in large-scale production of hemp plants [5]. Increased production led to a rise in the incidence and severity of different diseases of hemp plants, those of which are not reported previously. *Fusarium* spp. seem to cause significant economic losses in hemp production as it induces reduced raw material production, possible contamination by mycotoxin residues, as well as the possible

introduction of opportunistic pathogens to humans and animals [7].

In our greenhouse condition, hemp plants and seedlings developed wilting and damping-off symptoms, respectively. Isolation, identification, and pathogenicity test of the causal agent revealed that *Fusarium* spp. are related to the disease. Fusarium-induced wilt and damping-off diseases lead to the decay of germinating seeds and young seedlings, and whole plant collapse of adult plants, which represents one of the most important yield constraints in nursery and field environments [21]. Soil-borne *Fusarium* spp. is one of the most frequent pathogens causing damping-off in seedling plants [22]. The definition of damping-off is not straightforward in the literature. Many authors refer to damping-off as a "disease" [23], while others refer to damping-off as a "symptomatic condition" [21,24]. Damping-off is one of the most severe plant diseases and causes seed rot, root rot, and stem rot. According to the time of disease emergence, the symptoms can be divided into two stages: seed decay before emergence and whole-plant decay, wilt, and death after emergence [21]. In adult plants with different developmental stages, *Fusarium* spp. can cause wilt disease. Conidia of soil-borne *Fusarium* spp. germinates and penetrates through the cracks or wounds of roots [25–28]. The penetration process is likely enhanced by the vast array of middle lamella- and cell wall-degrading enzymes secreted by *Fusarium* spp. [29]. After the penetration, the rot cortex is colonized by emerging mycelia [30]. From the cortex, the hyphae penetrate the endodermis and invade the xylem vessels through the pits, those of which are relatively thinner portions of the cell wall that adjacent cells thus enabling communication or exchange of fluid through. Then, mycelia grow upward direction (colonization) of the stem and crown of the plants [24]. Inside the xylem vessels, the mycelia produce microconidia, which are released to travel upward in the transpiration stream, until trapped in pit cavities or at vessel end walls. They germinate into new hyphae and penetrate adjacent vessel elements to continue colonization and increase infection. Through these continuous infection cycles, *Fusarium* hyphae spread within the cell apoplast, which leads to significant symptom development [29]. Eventually, this induces wilting of lower branches, followed by the entire plant death.

We confirmed our identified fungal isolates AMCF1 and AMCF2 as *F. solani*, and AMCF3 as *F. proliferatum via* sequencing and phylogenetic tree analysis using the sequence of ITS, EF, and TUB genes. The *Fusarium* spp. are a widely spread phytopathogen identified in an extensive variety of hosts. The *Fusarium* genus is one of the most heterogeneous fungi and is difficult to classify. Currently, molecular approaches are proposed to be more sensitive than other methods for the identification of fungi, including the *Fusarium* spp. For these reasons, the molecular biological technique has been established in *Fusarium* systematics and the molecular variation at the DNA level has been investigated in numerous studies [31–33]. The use of polymerase chain reaction (PCR) with primers targeted to the internal transcribed spacer (ITS) region of the ribosomal DNA for the identification of the *Fusarium* spp. [34–36]. Elongation factor 1α (EF) plays an essential role in the translation of mRNA on ribosomes of eukaryotic fungal cells [37]. EF is functionally homologous to the prokaryotic factor EF-Tu and structurally conserved throughout evolution, and therefore it has been often used for phylogenetic studies [38]. The nucleotide sequence of the EF gene encoding a part of the protein translation machinery was first used in fungi in Fusarium [39]. The gene appears to be consistently single-copy and shows a high level of sequence polymorphism among related species so it was considered an alternative to rDNA [8] and to have desirable properties for phylogenetic inference in other groups of pathogenic fungi [40]. TUB belongs to the tubulin superfamily. They bind with each other through complex tubulin folding pathways to form β-tubulin heterodimers, which are major components of microtubules [41–43]. Microtubules can switch between growth and shrinkage, and this phenomenon is called dynamic instability, which is driven by GTP hydrolysis and essential for many microtubule-associated events, such as mitosis, vegetative growth (hyphae, conidia formation) [44–46]. The TUB gene sequence has been reported that it is an ideal marker for the analysis of deep-level phylogenies and for complex species groups, including *Fusarium* spp. [47]. Together with pathogenicity assay and molecular analysis, we suggest that *F. solani* AMCF1 and AMCF2, and *F. proliferatum* AMCF3 are causal agents of Fusarium wilt disease of hemp plants.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Promotion (No: 1425151434) of Innovative Businesses for Regulation-Free Special Zones funded by the Ministry of SMEs and Startups (MSS, Korea).

References

[1] Su FF, Yang G, Zheng YG. Cultivation and breeding of industrial *Cannabis sativa*. Zhongguo Zhong Yao Za Zhi. 2022;47(5):1190–1195.

[2] Yeasmin F, Choi HW. Natural salicylates and their roles in human health. Int J Mol Sci. 2020;21(23): 9049.

[3] Ahmed ATMF, Islam MZ, Mahmud MS, et al. Hemp as a potential raw material toward a sustainable world: a review. Heliyon. 2022;8(1): e08753.

[4] Rivedal HM, Funke CN, Frost KE. An overview of pathogens associated with biotic stresses in hemp crops in Oregon, 2019 to 2020. Plant Dis. 2022; 106(5):1334–1340.

[5] Punja ZK. Emerging diseases of *Cannabis sativa* and sustainable management. Pest Manag Sci. 2021;77(9):3857–3870.

[6] Olivain C, Humbert C, Nahalkova J, et al. Colonization of tomato root by pathogenic and nonpathogenic *Fusarium oxysporum* strains inoculated together and separately into the soil. Appl Environ Microbiol. 2006;72(2):1523–1531.

[7] Gwinn KD, Hansen Z, Akinrinlola R, et al. Diseases of *Cannabis sativa* caused by *Fusarium*. Front Agron. 2022;3:796062.

[8] O'Donnell K. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. Mycologia. 2000;92(5):919–938.

[9] Summerell BA. Resolving *Fusarium*: current status of the genus. Annu Rev Phytopathol. 2019;57: 323–339.

[10] Crous PW, Lombard L, Sandoval-Denis M, et al. *Fusarium*: more than a node or a foot-shaped basal cell. Stud Mycol. 2021;98:100116.

[11] Geiser DM, Al-Hatmi AM, Aoki T, et al. Phylogenomic analysis of a 55.1-kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. Phytopathology. 2021;111(7):1064–1079.

[12] Heo AY, Koo YM, Choi YJ, et al. First report of peach fruit rot caused by *Fusarium avenaceum* in korea. Res Plant Dis. 2020;26(1):48–52.

[13] Schneider CA, Rasband WS, Eliceiri KW. NIH image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9(7):671–675.

[14] White TJ, Bruns T, Lee S, et al. . Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York (NY): Academic Press, 1990. p. 315–322

[15] Choi HW, Ahsan SM. Biocontrol activity of *Aspergillus terreus* ANU-301 against two distinct plant diseases, tomato *Fusarium* wilt and potato soft rot. Plant Pathol J. 2022;38(1):33–45.

[16] Kumar S, Stecher G, Li M, et al. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(6): 1547–1549.

[17] Chehri K, Salleh B, Zakaria L. Morphological and phylogenetic analysis of *Fusarium solani* species complex in Malaysia. Microb Ecol. 2015;69(3): 457–471.

[18] Han K, Lee H, Park YM, et al. The first report of *Fusarium solani* causing wilting in *Cnidium officinale* in Korea. Res Plant Dis. 2021;27(2):76–78.

[19] Kim W, Choi H, Park G, et al. Fusarium wilt of Korean blackberry caused by *Fusarium cugenangense*. Res Plant Dis. 2021;27(4):187–191.

[20] Moura RD, de Castro LAM, Culik MP, et al. Culture medium for improved production of conidia for identification and systematic studies of *Fusarium* pathogens. J Microbiol Methods. 2020; 173:105915.

[21] Lamichhane JR, Dürr C, Schwanck AA, et al. Integrated management of damping-off diseases. A review. Agron Sustain Dev. 2017;37(2):10.

[22] Berg LE, Miller SS, Dornbusch MR, et al. Seed rot and damping-off of alfalfa in Minnesota caused by *Pythium* and *Fusarium* species. Plant Dis. 2017; 101(11):1860–1867.

[23] McNew GL. The nature, origin, and evolution of parasitism. Plant Pathol. 1960;2:19–69.

[24] Agrios GN. . Parasitism and disease development. In: Plant pathology. 5th ed. Burlington (MA): Elsevier Academic Press, 2005. p. 79–103.

[25] Inoue I, Namiki F, Tsuge T. Plant colonization by the vascular wilt fungus *Fusarium oxysporum* requires FOW1, a gene encoding a mitochondrial protein. Plant Cell. 2002;14(8):1869–1883.

[26] Rodríguez-Molina MC, Medina I, Torres-Vila LM, et al. Vascular colonization patterns in susceptible and resistant tomato cultivars inoculated with *Fusarium oxysporum* f. sp. *lycopersici* races 0 and 1. Plant Pathol. 2003;52(2):199–203.

[27] Mary Wanjiru W, Zhensheng K, Buchenauer H. Importance of cell wall degrading enzymes produced by *Fusarium graminearum* during infection of wheat heads. Eur J Plant Pathol. 2002;108(8): 803–810.

[28] Mandeel QA. Modeling competition for infection sites on roots by nonpathogenic strains of *Fusarium oxysporum*. Mycopathologia. 2007; 163(1):9–20.

[29] Walter S, Nicholson P, Doohan FM. Action and reaction of host and pathogen during *Fusarium* head blight disease. New Phytol. 2010;185(1): 54–66.

[30] Bishop CD, Cooper RM. An ultrastructural study of root invasion in three vascular wilt diseases. Physiol Plant Pathol. 1983;22(1):15–IN13.

[31] Arif M, Zaidi NW, Haq Q, et al. Morphological and comparative genomic analyses of pathogenic and non-pathogenic *Fusarium solani* isolated from *Dalbergia sissoo*. Mol Biol Rep. 2015;42(6): 1107–1122.

[32] Datta J, Lal N. Application of molecular markers for genetic discrimination of Fusarium wilt pathogen races affecting chickpea and pigeonpea in major regions of India. Cell Mol Biol. 2012;58(1): 55–65.

[33] Short DP, O'Donnell K, Geiser DM. Clonality, recombination, and hybridization in the plumbing-inhabiting human pathogen *Fusarium keratoplasticum* inferred from multilocus sequence typing. BMC Evol Biol. 2014;14(1):91.

[34] Dubey SC, Priyanka K, Singh V. Phylogenetic relationship between different race representative populations of *Fusarium oxysporum* f. sp. *ciceris* in respect of translation elongation factor-1α,

β-tubulin, and internal transcribed spacer region genes. Arch Microbiol. 2014;196(6):445–452.

[35] Chang SC, Macêdo DP, Souza-Motta CM, et al. Use of molecular markers to compare *Fusarium verticillioides* pathogenic strains isolated from plants and humans. Genet Mol Res. 2013;12(3): 2863–2875.

[36] Mirete S, Patiño B, Jurado M, et al. Structural variation and dynamics of the nuclear ribosomal intergenic spacer region in key members of the *Gibberella fujikuroi* species complex. Genome. 2013;56(4):205–213.

[37] Moldave K. Eukaryotic protein synthesis. Annu Rev Biochem. 1985;54(1):1109–1149.

[38] Kamaishi T, Hashimoto T, Nakamura Y, et al. Complete nucleotide sequences of the genes encoding translation elongation factors 1α and 2 from a microsporidian parasite, *Glugea plecoglossi*: implications for the deepest branching of eukaryotes. J Biochem. 1996;120(6):1095–1103.

[39] O'Donnell K, Kistler HC, Cigelnik E, et al. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci U S A. 1998;95(5):2044–2049.

[40] Roger AJ, Sandblom O, Doolittle WF, et al. An evaluation of elongation factor 1 alpha as a phylogenetic marker for eukaryotes. Mol Biol Evol. 1999;16(2):218–233.

[41] Nogales E. Structural insights into microtubule function. Annu Rev Biochem. 2000;69(1):277–302.

[42] Tian G, Huang MC, Parvari R, et al. Cryptic out-of-frame translational initiation of TBCE rescues tubulin formation in compound heterozygous HRD. Proc Natl Acad Sci USA. 2006;103(36):13491–13496.

[43] Tian G, Huang Y, Rommelaere H, et al. Pathway leading to correctly folded β-tubulin. Cell. 1996; 86(2):287–296.

[44] Mitchison T, Kirschner M. Dynamic instability of microtubule growth. Nature. 1984;312(5991): 237–242.

[45] Walker RA, O'brien ET, Pryer NK, et al. Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. J Cell Biol. 1988;107(4): 1437–1448.

[46] Zhu Y, Zhang Y, Duan Y, et al. Functional roles of α1-, α2-, β1-, and β2-Tubulins in vegetative growth, microtubule assembly, and sexual reproduction of *Fusarium graminearum*. Appl Environ Microbiol. 2021;87(20):e00967–e01021.

[47] Begerow D, Beate J, Oberwinkler F. Evolutionary relationships among β-tubulin gene sequences of basidiomycetous fungi. Mycol Res. 2004;108(11): 1257–1263.