Morphological and molecular characteristics of fungal species associated with crown rot of strawberry in South Korea

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Abstract

Background Crown and root rot is the most important and destructive strawberry diseases in Korea as it causes substantial economic loss. In August 2020, a severe outbreak of crown and root rot on strawberries (Fragaria × ananassa Duch.) was observed in the greenhouse at Sangju, South Korea. Infected plantlets displayed browning rot within the crown and root, stunted growth, and poor rooting.

Methods and results Thirty fungal isolates were obtained from the affected plantlet. Isolates were identified based on morphological characteristics and pathogenicity test as well as sequence data obtained from internal transcribed spacer, large subunit ribosomal ribonucleic acid, translation elongation factor, and RNA polymerase II-second largest subunit. Results showed that the crown and root rot of strawberry in Korea was caused by three distinct fungal species: Fusarium oxysporum f. sp. fragariae, F. solani, and Plectosphaerella cucumerina. To the best of our knowledge, F. solani, and P. cucumerina are reported for the first time as the causal agents of the crown and root rot of strawberry in South Korea. Pathogenicity tests confirmed that these isolates are pathogenic to strawberry.

Conclusions Understanding the composition and biology of the pathogen population will be helpful to provide effective control strategies for the disease.

Keywords Morphology · Multilocus phylogeny · Root rot · Strawberry

Introduction

Strawberry (Fragaria × ananassa) is cultivated across South Korea. South Korean strawberry is the most popular fruit due to its softness and sweetness. In South Korea, the cultivation of this fruit has increased rapidly in recent years. The cultivation area occupies 6435 hectares, and the total production is ~ 208,699 tons [1]. With the expansion of growing areas and increase in output, many fungal diseases are posing a challenge to strawberry growers in South Korea. Among them, the crown and root rot in strawberry is the most important and destructive disease. It is also a common threat to commercial production of strawberry worldwide, including Australia, Bangladesh, and China [2–5]. This disease limits plant growth and fruit production [2, 6]. The browning rot within the crown and root, stunted growth, and poor rooting are the typical manifestations in an infected plant [7]. Single or combinations of fungal and oomycete pathogens are responsible for this strawberry disease [2]. Fusarium oxysporum and Rhizoctonia spp. are the frequently reported fungal species that cause crown and root rot of strawberry [2, 8]. Thus far, in South Korea, Fusarium oxysporum f. sp. fragariae, Pythium spp. R. fragariae, R. solani, and Neopestalotiopsis clavispora have been reported as the so-called crown and root rot of strawberry [9, 10].

The causative agents of crown and root rot of strawberry were identified using the most traditional method [3, 9]. The shape and size of the conidia and chlamydospores and culture characteristics were used in the conventional method. These criteria are not enough to delineate fungal species, especially species within the Fusarium oxysporum complex due to the following reasons: the fungal species complex share similar features and environmental factors that influence morphological characteristics [11, 12]. Molecular analysis along with morphological characteristics is a highly recommended approach for the precious identification of fungal
species. Nam et al. [9] described the genetic diversity of *F. oxysporum* f. sp. *fragariae* from strawberry in Korea using the nuclear ribosomal intergenic spacer region and translation elongation factor (*EF-la*) gene. They found that all the isolates of *F. oxysporum* f. sp. *fragariae* were formed of three clades in the phylogenetic tree. *EF-la* is an essential molecular marker that identifies most but not all fusaria [6]. O’Donnell et al., [13] suggested DNA sequence-based identification of *Fusarium* using TEF-1, RNA polymerase II largest (RPB1), and the second largest subunit (RPB2) marker. *Fusarium* spp. needs to be identified using the recommended new gene marker.

According to the most of the grower from the Sangju region of South Korea, crown and root rot of strawberry has become more destructive and prevalent recently. The pathogens responsible for this disease in Sangju, Korea, have not been systematically investigated. Anxiety among the farmers is growing as the management strategies that were taken to contain this disease were not effective. One of the main reasons behind this could be the lack of proper diagnosis of the pathogen population. The sensitivity of fungi in different genera or different species within the same genus against a particular fungicide is not the same [14, 15]. Hellin et al. [14] found that *F. culorum* is more sensitive to triazoles compared with *F. graminearum* and *F. cerealis*. *Cholletotrichium truncatum* isolated from bell pepper is less susceptible to benomyl fungicide compared with *F. culorum* [15]. The identification of the pathogen population is essential to develop control strategies especially using fungicides.

This study aimed to identify the potential pathogens associated with the crown and root rot of strawberry in Sangju, Korea, characterized using a morphological and multigene approach. Moreover, this study aimed to confirm the pathogenicity of any recovered isolates.

**Materials and methods**

**Sample collection, pathogen isolation, and primary identification of pathogen population**

Twenty-five affected strawberry plants showing wilting, collapse and root and crown rot were sampled from five greenhouses in Sangju, South Korea (Fig. 1A–C). Crown was the more affected part compared with the root in these plants. The plants were propagated using the runner pegging method in a pot containing pasteurized soil and vermicompost. Plants were drip-irrigated with nutrient adjusted river water to adjust nutrients in the pot soil. Approximately 6 mm² tissue pieces were cut from the discolored area of the crown (Fig. 1D) of sampled plant, surface sterilized by dipping into 0.5% NaOCl solution for 1 min followed by 40 s in 70% ethanol, rinsed twice in sterile water, and then blot-dried with clean tissue paper. Tissue pieces plated on potato dextrose agar (PDA; Difco Becton Dickinson) were amended with 0.05 g/L tetracycline and incubated at room temperature (23 °C ± 2 °C) in the dark for 2–3 d. Hyphal tips of emerging hyphae were transferred to fresh PDA and were cultured for further use. A total of 30 pure fungal isolates were obtained and identified to genus level using morphological characteristics [16–19]. For a long time, all the isolates were preserved as mycelium in 10% glycerin at −70 °C. The fungal isolates from the strawberry plant were tentatively identified as *Fusarium* spp. and *Plectosphaerella* spp. based morphological criteria [17–19]. All the isolates of *Plectosphaerella* spp. shared the similar colony color, whereas some isolates of *Fusarium* spp. were pinkish-white and some were white. Four isolates *Plectosphaerella* spp. and nine isolates from *Fusarium* spp. (six pinkish-white and three white) were selected for species identification using the molecular approach. Actively growing cultures on PDA were used for DNA isolation. Genomic DNA was isolated using the HiGene Genomic DNA Prep Kit (BIOFACT, Yuseong-Gu, and Daejeon, Korea) following the instruction of the manufacturer and then stored at 4 °C for further use.

Following four genes were selected for amplification and sequencing: the internal transcribed spacer (*ITS*) region and domains D1/D2/ D3 of the nuclear large subunit (*LSU*) rRNA, the translation elongation factor 1 alpha (*EF-1 α*), and the second largest subunit (*rpb2*). The selected genetic fragments (*ITS*, *LSU*, *EF-1 α*, and *rpb2*) were amplified with the primers listed in supplementary Table 1 using the PCR-max Alpha cyclers (PCR max, Stone, United Kingdom) in a total volume of 25 uL. The PCR mixture contained 2.5 µL F-star buffer, 0.5 µL dNTP Mix (each 10 mM), 1 µL forward primer (10 pmol), 1 µL reverse primer (10 pmol), 1 µL template DNA, 0.2 µL F-star (*Taq* DNA polymerase), and 18.8 µL Invitrogen ultrapure DNase/RNase free distilled water. The PCR conditions for *ITS* were as follows: 4 min at 95 °C, 30 s at 95 °C, 30 s at 55 °C (annealing), 45 s at 72 °C (34 cycles), and a final extension of 7 min at 72 °C. The PCR conditions for *LSU*, *EF-1 α*, and *rpb2* were almost similar to that of *ITS* except for the annealing temperature. The annealing temperature for *LSU*, *EF-1 α*, and *rpb2* were 60 °C, 56 °C, and 59 °C, respectively. To confirm successful PCR reactions, all resulting PCR products were visualized in 1.5% agarose gels (wt/v) stained with ethidium bromide and viewed under ultra-violet light. The products of the confirmed PCR reaction were purified using a HiGene PCR Purification Kit (Yuseong-Gu) as instructed by the manufacturer and sequenced in both directions directly with the help of Macrogen, Inc (Seoul, Korea). The resulting sequences
were analyzed in SeqMan v.7.1 from the Lasergene package (DNASTAR, Inc. Madison, WI 53705 USA), edited manually whenever necessary, saved as a consensus sequence, and then deposited in the GenBank. The accession numbers of present and reference isolates are given in supplementary Table 2.

Multilocus phylogenetic analysis and species recognition

The obtained individual consensus sequences from *Fusarium* spp. were blasted against the Fusarium MLST database, and those obtained from *Plectosphaerella* spp. were blasted against GenBank to determine the closest matching species and species complex. Fungal species similar to the new isolates were used in phylogenetic analysis. Selected gene sequences of *Fusarium* spp. and *Plectosphaerella* spp. were downloaded from GenBank. All the fungal strain used in phylogenetic analysis are listed along with assigned GenBank accession numbers in supplementary Table 2. Individual gene sequences from the isolates belonging to the same genus were aligned using MEGA v.6.06 software [20]. The aligned sequences of Multiple genes/loci were concatenated in Mesquite v.2.75 [21]. First, the single alignment of *ITS, LSU, EF-1 α*, and *rpb2* genes of *Fusarium* spp. isolates were used for phylogenetic analysis using the Fusarium MLST database. Second, a concatenated sequence of *EF-1 α* and *rpb2* genes were used for phylogenetic inference based on two independent algorithms—Bayesian analyses (BI) and maximum likelihood (ML). Regarding *Plectosphaerella* spp. isolates, phylogenetic analysis based on BI and ML independent algorithms was conducted using a concatenated data set of *ITS, LSU, EF-1 α*, and *rpb2*. Mr Model Test v.2.3 [22] was used for the best fit model. BI was run in Mr

Fig. 1 Crown and root rot of strawberry; **A–B** Affected strawberry plants in green house; **C** Root and crown discoloration in wilted and collapse strawberry plant in green house; **D** Crown discoloration. (Color figure online)
Bayes v.3.2.2 and ML in MEGA v.6.0 [20, 23]. BI analysis included a Markov Chain Monte Carlo algorithm of four chains, with a stop rule option (split frequencies were below 0.01), and a tree was saved every 1000 generations. The 50% majority rule consensus trees and posterior probabilities (PP) were determined from the 75% held phylogenies after discarding 25% of the samples as the burn-in phase. The resulting trees were visualized using FigTree v.1.4.2 and MEGA v.6.0. For ML analyses, the bootstrap method (1000 replication), Tamura-Nei model, uniform rate, completed deletion and nearest-neighbor-interchange were used as default options.

**Morphological characterization**

Morphological characteristics of two representative isolates from each species were observed on PDA, oat-meal ager (OA), malt-extract agar (MA), corn-meal agar (CA), and V8 media after incubation at 25 °C under 16 h light/8 h dark cycle. The fungal block (5 mm) from the seven-day culture of each isolate was cut from the actively growing margin of the seven-day old culture on PDA, transferred on 90-mm petri dish and two perpendicular measurements of colony diameter were determined every day for five days. The size and shape of conidia, microconidia, macroconidia, chlamydospores, and conidiophores were examined and measured after mounting fungal structures slide glass in sterile water using light microscopy (Olympus BX43 microscope) at 400× magnification. The average and standard deviation were determined based on the measurement of 50 randomly selected conidia, microconidia, macroconidia, chlamydospores, and conidiophores each. Mycelial growth at various temperatures was evaluated. Mycelial block of approximate size 5×5 mm were cut from the actively growing margin of the seven-day old culture on PDA, transferred on 90-mm petri dish containing 25-ml PDA, and then incubated at 15 °C, 18 °C, 22 °C, 25 °C, 28 °C, 30 °C, 32 °C, and 35 °C in complete darkness. The single-trial consisted of three PDA plates per isolate per temperature. The colony diameter was measured as described above.

**Pathogenicity assay**

For the pathogenicity test, strawberry seedlings (cultivars) were collected from the greenhouse and transplanted in a pot containing autoclaved pit soils. After transplanting, seedlings were placed in a growth chamber at 25 °C and under 16 h/8 h light/dark cycle and were watered regularly. The runner came out from the established plant. New strawberry plants were propagated by pegging down plantlets at the end runners into pots containing autoclaved pit soils. The plantlets were cut to free from the mother plant when they started to grow new leaves after approximately one month and were used for pathogenicity test. After seven days of post-detaching from the mother plant, the plantlets were removed from pots, and the potting mixture was extracted from roots (shaking or with hand). Conidial suspensions were prepared in sterile distilled water for 20-day-old *Fusarium* spp. and 10-day-old *Plectosphaerella* spp. culture. Approximately 50 mL of water was poured into a culture plate, and the mycelium was scraped with a sterile glass rod and filtered through a double-folded cheesecloth. The conidial concentration in suspension was determined using haemocytometer and adjusted to 2×10⁶ conidia ml⁻¹. Then, 10 replicate plants per species/inoculum were inoculated by dipping the root up to the crown in 500 mL of conidial suspension (2×10⁶ conidia ml⁻¹) for 24 h in the dark. The same replicated control plants (10) per experiment were treated by dipping the root up to crown in 500-ml sterile deionized water for 24 h in the dark. After either inoculation or treatment, Strawberry plants were transplanted in the pot filled with the autoclaved potting mixture (pit soils: sand=4:2), maintained in a growth chamber at 25 °C and under 16 h/8 h light/dark cycle, and irrigated daily with sterile water. Plants were monitored frequently to discover symptomatic plantlets.

Affected plantlets were further evaluated by observing the presence of root rot and discoloration inside the crown. Subsequently, the pathogen was reisolated and reidentified based on morphological characteristics and the sequence data of *rpβ2* gene. Disease incidence (DI) was estimated as the proportion of symptomatic plantlets compared with the total number of inoculated plantlets.

**Statistical analyses**

The optimum temperature for the mycelial growth rate of the identified fungal species was estimated via nonlinear regression analysis using Gaussian four-parameter process in SigmaPlot v.14 (Systat Software, San Jose, CA). The mean and standard deviation (SD) of data were estimated using MS Excel. The growth rate of mycelium on various culture media was analyzed via ANOVA using SAS v.9.4, and lsd test was used to test for significant differences among or between different treatments at \( P < 0.05 \).

**Results**

**Fungal isolation**

Thirty isolates were obtained from rotten tissue of the crown and root of ~25 affected strawberry plants collected from different greenhouses in Sangju, South Korea. Most of these isolates were *Fusarium* spp. (22 isolates) and the rest were *Plectosphaerella* spp. (eight isolates). Among the isolates belonging to *Fusarium* spp., the colony of majority isolates
was pinkish-white (16 isolates), whereas the colony of remaining six isolates were creamy or off white.

**Multilocus phylogenetic analysis**

The multigene sequences from *Fusarium* spp. and *Plectosphaerella* spp. were subjected to phylogenetic analysis separately. The first phylogenetic tree illustrates the phylogenetic analysis among the *Fusarium* species, and the second one was among the *Plectosphaerella* spp. As the sequences of ITS, LSU, EF-1α, and rpb2 were not available for all reference isolates, separate phylogenetic analysis was conducted using individual sequence data. In this case, unique gene sequences of reference *Fusarium* spp. were obtained from Fusarium MLST and GeneBank database. The present isolates were clustered into two separate clades (*F. oxysporum* species complex and *F. solani* species complex) consistently in the individual pyogenesis. The sequences of EF-1α, and rpb2 were believed to be more informative for *Fusarium* spp. identification. Thus, the concatenated sequence of EF-1α, and rpb2 were used to construct the multigene phylogenetic tree. Thirty-one representative *Fusarium* isolates, including the isolates from the current study and *Brunneochlamydosporium nepalense* as outgroup, were used to build the phylogenetic tree based on EF-1α, and rpb2 sequence data. The concatenated sequences data set comprised 1715 total characters, including gap. In BI, 50% majority rules consensus tree with PP (Fig. 2) was estimated from the remaining 11,178 trees after 3677 trees were discarded as the “burn-in” phase. Maximum likelihood analysis based on the Tamura-Nei model yielded the best ML tree with maximum log-likelihood of −6683.4999. Codon positions included were 1st + 2nd + 3rd + noncoding, and there were a total of 1198 places in the final dataset. Phylogenetic inference shows the position of all selected *Fusarium* isolate (nine isolates) (Fig. 2). Of these nine isolates, six were clustered with *F. oxysporum* f. sp. fragariae within the *F. oxysporum* species complex and three with *F. solani* within the *F. solani* species complex.

For the identification of *Plectosphaerella* spp., the multigene phylogenetic tree was constructed using the concatenated sequences of ITS, LSU, EF-1α, and rpb2. The analysis involved 21 representatives *Plectosphaerella* isolates, including the isolates from the current study and one *Brunneochlamydosporium nepalense* CBS 277 89 (used as outgroup). The concatenated sequences data set consists of 2400 characteristics, including gaps. The BI ended after 1 million generation, 50% majority rules

![Fig. 2](image-url) A Bayesian inference phylogenetic tree based on combined EF1-α and rpb2 sequence alignment of *Fusarium* spp. The present isolates are indicated in bold color. Bayesian posterior probability (PP ≥ 0.50), maximum likelihood bootstrap support values (ML ≥ 50) are shown at the nodes (PP/ML). The scale bar shows the expected number of substitutions per site. (Color figure online)
consensus tree with PP (Fig. 3) were estimated from 1085 trees left after the 272 trees were discarded as the burn-in phase. Maximum likelihood analysis yielded the best ML tree with maximum log-likelihood of \(-6629.1611\). The ML alignment matrix had 2307 positions in the final dataset, and the coding positions included were 1st + 2nd + 3rd + noncoding. The Tamura-Nei model was used to run the full likelihood analysis. The phylogenetic tree revealed the work of the isolates obtained in this study (Fig. 3). All the isolates clustered with reference isolates of \(P. cucumerina\). The clade of \(P. cucumerina\) is highly supported by the posterior probability and bootstrap value (PP = 1; BS = 98%).

**Morphological characterization**

Based on the molecular analysis, it was confirmed that the isolates obtained from the affected strawberry with crown and root rot from the five greenhouse belong to \(F. oxysporum\) f. sp. fragariae, \(F. solani\), and \(P. cucumerina\). The morphological characteristics of representative isolate of identified fungal species described in detail.

**Morphological characterization of \(F. oxysporum\) f. sp. fragariae isolate**

The colony and culture of \(F. oxysporum\) f. sp. fragariae isolate are shown in Fig. 4. The comparison of morphological characteristics of present and reference isolates of \(F. oxysporum\) f. sp. fragariae shown in Supplementary Table 3. The colony of present isolate (SJB593) on PDA was white and purple, whereas the colony of reference isolate (S-1) was white and light pink. The shape of microconidia of the present isolate match those of reference isolate described by Cho and Moon [24]. The present isolate produced cylindrical microconidia, whereas reference isolate produced ellipsoidal to falcate microconidia. The average size microconidia \((11.2 \times 3.9 \, \mu m)\) of the present isolate were slightly smaller than that microconidium \((12.2 \times 5.1 \, \mu m)\) of reference isolate. The average size of macroconidia of the present isolate was \(26.1 \times 4.7 \, \mu m\), which is also smaller than that of macroconidia \((43 \times 7 \, \mu m)\) of reference isolate.

**Morphological characterization of \(F. solani\) isolate**

Colony and microscopic characteristics of present isolate belonging to \(F. solani\) species complexes are shown in Fig. 4. The morphological characteristics, including colony

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**Fig. 3** A Bayesian inference phylogenetic tree based on combined ITS, LSU, EF1-\(\alpha\), and rpb2 sequence alignment of *Plectosphaerella* spp. The present isolates are indicated in bold color. Bayesian posterior probability (PP \(\geq 0.50\)), maximum likelihood bootstrap support values (ML \(\geq 50\)) are shown at the nodes (PP/ML). The scale bar shows the expected number of substitutions per site. (Color figure online)
color, shape, and size of microconidia, macroconidia, and chlamydospore of presents isolate, were comparable with those of reference isolate described by Chang et al. [25] (Supplementary Table 3). The colony color of the present isolate (SJB222) was white from up and creamy white from the reverse side, whereas Chang et al. [25] described the colony color of the isolate (Yadou7-6) as a pale yellowish and yellowish on the back. The shape of macroconidia produced in present isolates is consistent with the shape of macroconidia of reference isolate. The present isolates had bigger macroconidia (33.0 × 6.5 μm) than that of the reference isolate. Miroconida of present isolates were cylindrical, curve, or straight and were 9.7–17.3 × 3.4–8.3 μm in diameter. The chlamydospore produced by the present isolate were globose, pair form, and were 6.8–10.9 × 6.6–11.6 μm in diameter.

**Morphological characterization of *P. cucumerina*** isolate

The colony and culture of *P. cucumerina* are shown in Fig. 5. The comparison of morphological characteristics of present and reference isolate of *P. cucumerina* are shown in supplementary Table 4. The morphological characteristics of the present isolate are comparable with that of the reference isolate of *P. cucumerina* (CBS131739) described by Carlucci et al. [26]. The colony of the present isolate was creamy white and pale yellowish from the reverse side. Conidiophores were simple (rarely branched) and were 10.6–44.9 × 2.2–5.4 μm in diameter. Conidia were ellipsoidal, tapered or rounded ends, guttate, 0–1 septate, and 8.5–13.1 × 3.2–6.4 μm in diameter. On average, the conidial size of present isolates was bigger than that of reference isolates. Carlucci et al. [26] described the conidial size of *P. cucumerina* (CBS131739) as 7–10.5 × 2.5–3.5 μm (mean ± SD = 8.8 ± 1.3 × 2.8 ± 0.4 μm) in diameter.

**Effect of culture media and temperature on mycelial growth**

The media have a significant influence on the mycelial growth rate (Supplementary Fig. 1). The isolates of *F. solani* showed the highest growth rate on PDA, MEA, V8 and the least growth rate on CMA. In contrast, the isolates of *F. oxysporum f. sp. fragariae* showed the best growth rate on V8 followed by CMA, OMA and the least growth rate on MEA. The growth rate of the isolate of *P. cucumerina* was significantly higher on V8 and CMA than on PDA, MEA, and OMA (Supplementary Fig. 1A). The mycelium growth rate on the same culture media also depends on fungal species (Supplementary Fig. 1B). For example, *F. oxysporum f. sp. fragariae* grew significantly
well on OMA followed by *F. solani* and *P. cucumerina*, whereas *F. solani* grew significantly better on MEA followed by *F. oxysporum f. sp. fragariae* and *P. cucumerina*. *Plectrosporum cucumerina* showed poor growth rate on all tested media compared with that of *F. solani* and *F. oxysporum f. sp. fragariae* (Supplementary Fig. 1B). The selected isolates representing each of the three fungal species were grown on PDA at 15 °C–35 °C. The maximum growth rate of *F. solani* (isolate SJB222) was estimated at 25.2 °C. In contrast, the top growth rates of *F. oxysporum f. sp. fragariae* (isolate SJB593) and *P. cucumerina* (isolate SJB271) were 22.0 °C and 22.7 °C, respectively (Supplementary Fig. 2). *Plectrosporum cucumerina* exhibited the slowest growth rate at all tested temperature regimes. *F. solani* (isolate SJB222) showed the highest growth rate at higher temperature (22.5 °C–35 °C), whereas *F. oxysporum f. sp. fragariae* showed at lower temperature (15 °C–22.5 °C).

**Pathogenicity test**

To confirm Koch’s postulates, the pathogenicity of an identified representative isolate of *F. oxysporum* (SJB593), *F. solani* (SJB222), and *P. cucumerina* (SJB271) were tested by following the root dip assay method. The pant showed typical crown and root rot symptoms after 15 (*Fusarium* spp.) and 18 (*P. cucumerina*) days of inoculation (Fig. 6). The symptoms include wilting, root rot, and discoloration inside the crown, which was similar to those observed naturally infected plantlet in greenhouse. But tested isolate showed a different level of aggressiveness intern of DIs. The hermit of *P. cucumerina* showed the highest virulence with 80% DI followed by *F. solani* with 70% and *F. oxysporum f. sp. fragariae* with 60% DI. The control plant remains healthy and asymptomatic (Fig. 6).
The crown and root rot disease is a significant threat to strawberry production worldwide [8]. According to the grower at Sangju, South Korea, the level of the crown and root rot in strawberry greenhouse increase in the late production season. Low pH levels (4.5–6) influence the outbreak of this disease [8]. Three fungal species were recovered from the deceased strawberry crown and were discerned as *F. oxysporum f. sp. fragariae*, *F. solani*, and *P. cucumerina* based on distinct molecular and morphological characteristics. The pathogenicity assay revealed that the isolates of these fungal species reproduced identical disease symptoms. Therefore, this study concluded *F. oxysporum f. sp. fragariae*, *F. solani*, and *P. cucumerina* are responsible for the crown and root rot of strawberry in Sangju, South Korea. *Fusarium oxysporum f. sp. fragariae* were the most reported fungal species that cause crown and root rot of strawberry, but this is the first report showing *F. solani* and *P. cucumerina* as the causative agent of this disease. Proper diagnosis of this new pathogen is crucial because early identification and disease monitoring are the most important critical steps for implementing any disease management program [27].

With the progress in molecular analysis and bioinformatics, the crystal explicit knowledge regarding fungi and their association with the host has increased over the last decade [28]. The multigene phylogenetic approach uses sophisticated and reliable tools for the classification of cryptic fungal taxa and fungi, including plant pathogen. The filamentous fungal genus *Fusarium* is one of the most destructive plant pathogen comprised of 300 phylogenetically distinct species distributed among ~23 species complex [13, 30]. Initially, fungal species within the *Fusarium* genus were identified based on iconic fusiform multiseptate macroconidia. The morphological and morphological phylogenetic studies revealed that they convergently evolved into different lineages of ascomycetes [13]. So, the use of molecular markers (DNA sequences) is more reliable for identifying closely related species. In this study, we identified *F. oxysporum* and *F. solani* species based on phylogenetic inference using concatenated sequences of *EF-1 α*, and *rpb2*.

*Plectosporium* was first described in 1995 as the new genus for the species previously known as *Fusarium tabacinum* (Cephalosporium tabacinum), the anamorph of *Plectosphaerella cucumerina* [29]. Some studies recommend using LSU, ITS, EF1α, and RPB2 sequences data to resolve the taxonomy of the *Plectosphaerella* species Giraldo and Crous 2019, [19]. This study also found that the concatenated sequences of these loci are very informative for identifying *P. cucumerina*.

The morphological characteristics of identified fungal species in this study were comparable with that of the respective reference species and species complex (Supplementary Tables 3 and 4). This study found that there are some differences in morphological characteristics; for example, macroconidia of the present isolate of *F. oxysporum f. sp. fragariae*(SJB 593) were smaller than that of reference isolate (S-1), whereas macroconidia of the present isolate of
F. solani (SJB222) were larger than that of reference isolate Yadou7-6 (Supplementary Table 3). The conidia of P. Cucumerina also isolates are larger than that of reference isolate CBS 131,739. The slight morphological differences within the same species are explained by the difference in host, geographical location, and cultural condition. Many studies reported that morphological characteristics fungal species are influenced by the host, geographical location, culture media, and cultural condition [11, 31, 32].

This study also found that F. oxysporum f. sp. fragariae species grew well on PDA and V8 and F. solani and P. cucumerina on V8 and CMA. So, V8 could be the best choice to culture these species.

Among the fungal species, Fusarium species exhibited greater mycelial growth in warm temperature (Supplementary Fig. 2). The adaptability of Fusarium species in warm temperature explain its current geographical distribution. Warm and rainfall are the favorable condition for Fusarium species to infect crops [33]. In contrast, the favorable condition for P. cucumerina were high humidity and low temperature (20 °C−26 °C) [34].

In general, both Fusarium species (F. oxysporum f. sp. fragariae and F. solani) soil in-habiting species capable of causing disease, including wilt, root rot and crown rot of all economically important crops. Fusarium oxysporum f. sp. fragariae is the commonly reported pathogen associated with crown rot of strawberry across the world including korea [24, 35−37]. In South Korea, F. solani has been reported as the causative agent of wilt of cucumber, coastal hog fennel and sweet potato [38–40]. Fusarium solani also has been reported as a pathogen causing crown and root rot on strawberry in Spain [41]. To the best of our knowledge, F. solani has not been reported as the causative agent of crown and root rot of strawberry in South Korea.

Like Fusarium spp., P. cucumerina also soil inhabiting plant pathogen and has been reported on many crops, including alfalfa, cabbage, potato, tomato, and sunflower [42, 43]. Though having a wide host range, it was reported only on the wild arrowhead in South Korea [43]. To the best of our knowledge, this is the first report of root and crown rot of strawberry caused by P. cucumerina in South Korea.

Evolving of the new pathogen under existing cultural practices and control methods indicates that it is necessary to adopt new cultural practices and control strategies. Strawberry grower in Sangju use river water for irrigation. Inoculum of this disease may come through contaminated irrigated water. Plant debris of affected plant also a crucial source of inoculum. Plant debris was observed during the survey. This plant debris may be mixed with soil in pot mixture and infect newly propagated plantlet. Proper disposal of affected plant debris is necessary. For developing chemical strategies to control this disease, a representative isolate of identified fungal taxa in this study needs to be tested for estimation of efficacy of traditional fungicides (benomyl, captan, mefenoxam and thiophanate methyl).

In conclusion, this study is the first detailed report of crown and root rot of strawberry in South Korea and the finding of this study indicates essential information to develop sustainable control measures for crown and root rot of strawberry in South Korea. This study sampled causal agents of crown and root rot of strawberry from only one area (Sangju) of Korea. It is very important to isolate fungal species associate with this disease to mitigate the risk to the strawberry industry in Korea. Accurate and rapid molecular diagnostic tools (PCR, qPCR) need to be developed.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06841-9.

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Data availability Resulting sequences have been deposited to GenBank, with accession numbers provided in Supplementary Table 2.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Animals rights There was no involvement of Human Participants and/or Animals in this research.

Informed consent Not applicable.

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