Identification and pathogenicity of species isolated from stored potato tubers showing symptoms of dry rot disease

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Abstract

Arises during the storage of potatoes as a result of the pathogens introduced during their growing and harvest. In this study, the causative bacteria of domestic potato dry rot disease were identified and their pathogenicity was confirmed. A total of 76 species were isolated from 93 potato necropods collected from samples inoculate with dry rot disease (Table 1). These 76 isolates were identified as *Fusarium boothii*, *F. circinatum*, *F. citricola*, *F. foetens*, *F. iranicum*, *F. longifundum, F. oxysporum, F. pseudoanthophilum F. solani, Botryotinia ranunculi*, *Clonostachys rosea*, and *Humicola nigrescens*. The average size of the inoculation site was ≥ 4.6 mm in *F. oxysporum* and *F. solani*, which were pathogenic to dry rot in potatoes but not as other strains for up to five weeks. The pathogenicity of *F. foetens* and *F. pseudoanthophilum* was related to high a molecular statistical flexibility by forming a single system with *F. oxysporum.* However, except for *F. oxysporum* and *F. solani*, these strains have not yet been reported to be associated with dry rot disease. Additionally, the length of the cross-section and longitudinal section of the potato sclera inoculated with *C. rosea* increased the most among all strains. This suggests that *C. rosea* is the dominant species involved in domestic potato dry rot disease. By contrast, there are no reports of the involvement of *B. ranunculi* and *H. nigrescens* in dry rot disease. Therefore, these strains can be seen as parasitic using potato sclerosis as nutrients in *in vivo* experiments through wounds and are not directly related to dry rot disease.

Introduction

Potatoes belong to the Solanaceae family, with approximately 230 species (species) found in the natural ecosystem, among which approximately 160 species form sclerosis (Kwon et al., 2005). Today, *Solanum tuberosum* is grown worldwide, with approximately 388 million tons of the tuber produced annually across 130 countries (Kim, 2020), 690,419 tons of which were produced in Korea in 2019 (KOSIS, 2021). As the highest source of energy and production per unit area among the world's major cultivated crops, potato production is expected to continue increasing (RDA, 2020). However, potatoes are vulnerable to a variety of diseases when stored for long periods of time. For example, losses caused by dry rot disease are estimated to range from 6 to 25% worldwide, with losses of up to 60% during long-term storage periods (Desjardins, 2006; Secor and Salas, 2001). The estimated economic damage of crops lost in the United States, the largest potato producer, ranges from $100 to $250 million per year (Slinger and Schisler, 2002), and accounts for up to 88% of the total post-harvest losses of potatoes in Gansu, China (He et al., 2004). Despite a lack of accurate statistics or reports, most potatoes with pathological disorders during potato storage in Korea have been identified as having dry rot disease (data not shown).

Dry rot disease is caused by the intrusion of various Fusarium species through wounds on the surface of potatoes during their harvest (Nelson et al., 1981). This pathogen invades potatoes through the soil and seeds and causes crop loss during storage (Tiwari et al., 2020). Starting as a small brown lesion, potato dry rot disease gradually expands, developing into a concentric dry region around dead tissue (RDA, 2020). Subsequently, the brown wrinkled tissue is discolored into black and can be distinguished with the naked eye. The lesion of the wrinkled tissue produces creamy white, pink, or orange spores and mycelium

during long-term storage at temperatures ranging from 5°C to 30°C (Bojanowski et al., 2013; Elsherbiny et al., 2016).

Dry rot caused by *Fusarium* occurs in all potato cultivation areas, and the distribution of *Fusarium* species varies depending on the cultivation time and geographical location (Tiwari et al., 2020). More than 13 species, including *F. acuminatum, F. avenaceum, F. crookwellense, F. culmorum, F. equiseti, F. graminearum, F. oxysporum, F. sambucinum, F. scirpi, F. semitectum, F. solani, F. sporotrichioides*, and *F. tricinctum*, are involved in the dry rot disease of potatoes worldwide (Cullen, 2005). The dominant species that cause dry rot in potatoes are *F. sambucinum* and *F. graminearum* in North America, *F. sambucinum* in Europe, *F. solani* in the United Kingdom, *F. solani* and *F. oxysporum* in South Africa, and *F. sambucinum* in China (Tiwari et al., 2020). Until 2000, *F. solani* was suggested as a bacterium that caused potato dry rot disease in Korea, and *F. oxysporum* was introduced as a pathogen that caused withering diseases in various crops; however, this was not explained in connection with potato dry rot disease (Kim, 2000; Park et al., 1988). Currently, dry rot disease pathogens in potatoes are registered as *Fusarium* spp. in the National Agricultural Products Disease and Pest Management System, and various *Fusarium* spp. have been reported to cause the disease (NCPMS, 2023). In addition, one study showed that *Clonostachys rosea*, also known as *Gliocladium roseum*, is not *Fusarium* but is involved in dry rot disease and simultaneously acts as an antagonist to *F. solani* and *F. oxysporum* (Theron and Holz, 1991).

Research has been conducted in North America, Europe, the United Kingdom, South Africa, and China to identify the dominant species of pathogens causing potato dry rot. However, few studies have identified the causative bacteria of dry rot disease during storage or reported the dominant species in Korea. Therefore, this study was conducted to identify and confirm the pathogenicity of the causative pathogens of domestic potato dry rot disease in Korea.

Materials and methods

Collection of pathogens for dry rot of potatoes

Tubers were collected from potatoes stored in traditional potato warehouses located on highland areas, including Wangsan-myeon and Daegwallyeong-myeon, and located on plain area, including Gangneung Wonju University's farm and Sacheon-myeon in Gangwon Province. The potato varieties were included Atlantic, Chubaek, Doobak, and Superior. The collected necrotic potato pathogens were stored in a 20°C incubator at Gangneung Wonju University's Plant Pathology Laboratory for three days to induce growth.

Separation and identification of pathogens

Dry rot pathogens were isolated from the plant pathology laboratory of Gangneung Wonju University using *Solannum tuberosum* sclera with signs of disease. To separate pathogens, fragments, including the surface of the potato sclera containing the boundary of the disease, were prepared by cutting into 1-cm diameter slices. The potato slices were sterilized in 70% ethanol for 30 s and 1% sodium hypochlorite (NaOCl) for 2 min on a clean bench, washed once with sterilized water, and dried on a sterilized filter

paper for 30 min. One dried fragment from the infected sclera was placed in 10 WA (water agar medium) and cultured for three days in a 25℃ incubator (Fig. 1). Mycelia of pathogens grown in potato fragments were cultured in a 20°C incubator for seven days by cutting the tip of the pathogen for pure separation and denting on potato dextrose agar (PDA) medium. Each fungus was separated from the potato agar medium after confirming the shape of mycelial growth, and the separated samples were analyzed using the ITS1/ITS4 primer pair. The system water was manufactured by the neighbor-joining method using MEGA7.

Pathogenicity test of dried potato rot strain

The potatoes used in the pathogenicity test were superior varieties grown in Wangsan-myeon, Gangneung-si, and Gangwon-do. The 12 identified strains were used as inoculators. The potato samples were immersed in 1% NaOCl for 30 min, washed three times with sterile water after sterilization, and dried for 30 min. The inoculator prepared 12 identified pathogens by implanting them in PDA and culturing them in an incubator at 20°C for 7 days. For the treatment of the inoculum, a 2-mm-deep hole was made in the base of the potato necrosis using a ø8mm Cork Borer, and the inoculum was cut and inserted with a ø10mm Cork Borer. Potatoes inoculated with the identified bacteria were separated and cultured in a sealed plastic container to prevent air contamination by pathogens during culturing. The invasion and pathogenicity of pathogens through wounds were investigated by placing the fungally inoculated sclera in a humidified translucent plastic box and maintaining a relative humidity of 99% at room temperature of 15–20°C.

The inoculation site and pathogenicity were visually checked at intervals of seven days after inoculation, and the length of disease spread was measured in the cross-section (Fig. 2) and longitudinal section (Fig. 3) of the potato sclera at five weeks after inoculation. Each treatment port, including the control port, was performed five times based on 13 unusual diameters. Statistical analysis was performed using SPSS (IBM SPSS Statistics for Windows version 26.0 (IBM Corp., Armonk, NY, USA) to analyze ANOVA, and the significance of each treatment was verified at $P < 0.05$ using Duncan's multiple range test (DMRT).

Results and discussion

Collection of pathogens for dry rot in potatoes

The total number of potatoes with dry rot disease after storage was 93, including 76 in Gangneung plain, 3 in Pyeongchang, 11 in Gangneung highland. To collect individuals with dry rot disease from potatoes, potato-only storage was investigated, and most potato objects with external diseases were infected with dry rot disease. There were a few cases of infection during storage, and it is believed that the spread of the disease was suppressed by screening and curing after harvest. Dry rot disease that occurred in certain varieties, such as Atlantic, Beckjak, Chubaek, Dano, Doobak, Jayoung, Superior, Wangsan, and showed typical symptoms of disease regardless of the variety (Fig. 4).

Separation and identification of pathogens

Of the 93 potatoes harboring dry rot disease, some had duplicate or separate fungal strains. As such, a total of 76 were ultimately isolated (Table 1). After analyzing the sequence of the ITS region using the ITS1/ITS4 primer pair in the 76 separate fungal strains, a total of 12 strains were analyzed, including nine from *Fusarium*, one from *Botryotinia*, one from *Clonostachys*, and one from *Humicola*.

Table 1
Type and number of fungi isolated from 93 tubers showing symptoms of dry rot disease (2021–2022).

Fungal species	No. of isolates
Fusarium boothii	8
Fusarium circinatum	5
Fusarium citricola	14
Fusarium foetens	16
Fusarium iranicum	5
Fusarium longifundum	2
Fusarium oxysporum	1
Fusarium pseudoanthophilum	4
Fusarium solani	13
Botryotinia ranunculi	2
Clonostachys rosea	4
Humicola nigrescens	2
Total	76

Table 2
Growth estimation based on the length of transverse and longitudinal sections to verify the pathogenicity of dry rot disease in potato tubers inoculated by 12 fungi collected in this study.

Treatment (inoculated fungi)	Longitudinal section (mm)	Transverse section (mm)
Control	0.00 c	0.00 d[z]
Fusarium boothii	3.46 b	3.50 c
Fusarium circinatum	3.68 b	5.84 b
Fusarium citricola	4.84 b	5.44 bc
Fusarium foetens	3.91 b	4.56 bc
Fusarium iranicum	3.13 b	3.66 c
Fusarium longifundum	3.06 b	4.2 bc
Fusarium oxysporum	2.93 b	4.94 bc
Fusarium pseudoanthophilum	3.12 b	3.8 bc
Fusarium solani	3.34 b	4.62 bc
Botryotinia ranunculi	2.93 b	3.36 c
Clonostachys rosea	6.90 a	11.48 a
Humicola nigrescens	3.05 b	4.28 bc

[z]Mean separation within columns by Duncan's multiple ranges test at $P \leq 0.05$.

The nine strains of the *Fusarium* spp. were *F. boothii*, *F. circinatum*, *F. citricola*, *F. foetens*, *F. iranicum*, *F. longifundum, F. oxysporum, F. pseudoanthophilum*, and *F. solani.* Among the *Fusarium*, the most frequently isolated strains were *F. foetens* (16), *F. citricola* (14), and *F. solani*. Other *Fusarium* strains were separated in the following order: *F. boothii*, *F. circinatum*, *F. iranicum*, *F. pseudoanthophilum, F. longifundum*, and *F. oxysporum.* The *Fusarium* strains involved in dry rot disease, which are most commonly found in domestic potato stores, are believed to be *F. foetens*, *F. citricola*, and *F. solani*. The remaining strains were identified as *Botryotinia ranunculi*, *Clonostachys rosea*, and *Humicola nigrescens* (Fig. 5). Among these, *F. solani, F. oxysporum*, and *C. rosea* have been reported to cause dry rot disease (Bojanowski et al., 2013; Theron and Holz, 1991). *C. rosea* is involved in dry rot disease and simultaneously acts as an antagonist of *F. solani* and *F. oxysporum* (Theron and Holz, 1991), although there have been no reports of any other identified pathogens associated with dry rot disease. The following have been reported to be representative of diseases caused by each pathogen: *F. boothii* has been reported to be associated with corn ear rot, pecan malady (Gryzenhout et al., 2016) and wheat red mold disease (Wegulo et al., 2018); F. *circinatum* causes pine branch disease (Woo et al., 2011) and pine

pitch canker (Maphosa, 2016); *F. citricola* causes tangerine branch ulcer disease (Sandoval-Denis et al., 2018); F. *foetens* is pathogenic to begonia (Schroers et al., 2004) and has been associated with the occurrence of Louis Vos Zalok disease (Lamprecht and Tewoldemedhin, 2017); *F. iranicum* has been identified in mushroom (Torbati et al., 2019) and wheat head light (Senatore et al., 2021); F. *longifundum* causes brown spots on the leaves of wild leafy vegetables (Matić et al., 2020); F. *pseudoanthophilum* is highly pathogenic in corn stalks in western Iran (Chehri et al., 2010) and is associated with *Fusarium* crown rot (Kazan and Gardiner, 2018); *B. ranunculi* is a complete generation of *Botrytis* species, a plant pathogen that causes damage to economically important crops worldwide and is pathogenic to Ranunculaceae, which are distributed in warm and cold regions (Staats et al., 2005); H. *nigrescens* is a soil by-product of potato packaging and is known to be pathogenic to plants (UAMH, 2023).

F. solani, generally known as the main cause of dry rot disease, was found to be far from related among *Fusarium* sp. in the system tree (Fig. 6), which showed a close relationship between 12 strains as a result of identification. *F. oxysporum* (Kwon et al., 2005) is considered another major cause of dry rot disease in Korea and exhibits a high molecular statistical flexibility through the formation of a single system with *F. foetens, F. pseudoanthophilum*, and *F. circinatum*. In this study, *F. acuminatum, F. avenaceum, F. crookwellense, F. culmorum, F. equiseti, F. sambucinum, F. scirpi, F. semitectum, F. sporotrichioides*, and *F. tricinctum* were not investigated in *Fusarium* (Cullen, 2005). *F. sambucinum* (Tiwari et al., 2020), known as a common or dominant species in North America, Europe, and China, was not identified among the isolated strains. These results suggest that *F. sambucinum* is not common in Korea among the various types of *Fusarium* strains that do not cause dry rot disease.

Pathogenicity test of dried potato rot strain

A pathogenic test of the 12 strains identified separately showed no signs of disease in the control potatoes, while all potatoes inoculated with the 12 strains *in vivo* into the sclera of the Sumi variety showed dry rot disease with a cross-sectional length of ≥ 3.5 mm and a longitudinal length of ≥ 2.9 mm. The average size of the inoculation site was ≥ 4.6 mm in cross-sectional length in the *F. oxysporum* and *F. solani* treatment zones, which are known to be involved in potato dried rot in Korea. When comparing the cross-sectional length of the two strains known as the main bacteria of dry rot disease with 3.8–5.4 mm, the cross-sectional length of *F. citricola, F. foetens, F. longifundum*, and *F. pseudoanthophilum*, pathogenicity was found to have similar diseases with no statistically significant difference. However, *F. oxysporum* and *F. solani* had pathogenic properties of dry rot disease in potatoes, but were not as pathogenic as other strains until 5 weeks in a suitable environment (15–20℃, 99% RH). It is possible that the *F. oxysporum* and *F. solani* strains were used after more than three series of cultures, which reduced their vitality. In addition, the optimal mycelium growth temperature of *F. oxysporum* and *F. solani* is 24–25℃ (Ezrari et al., 2021; Hibar et al., 2006; Jeon et al., 2013), and it is believed that mycelium growth was relatively inactive at 15–20℃, which is the condition used in this experiment. Among *Fusarium*-based pathogens, *F. foetens* and *F. pseudoanthophilum* are known to exhibit high molecular statistical flexibility through their formation of a single system with *F. oxysporum*. However, strains other than *F. oxysporum* and *F. solani* have not yet been reported to be associated with dry rot disease, and there is a need to

determine whether these strains develop dry rot disease. The cross-sectional length of the potato sclera inoculated with *C. rosea* was 11.48 mm and the longitudinal length was 6.9 mm, which represents the greatest disease growth among all strains. The length of the longitudinal section of all strains used in the experiment was approximately 2.9–4.8 mm in other treatment areas except for potatoes inoculated with *C. rosea*, and there was no statistically significant difference. The results of this study are similar to those of Theron and Holz (1991), who reported that *C. rosea* is also involved in dry rot disease, and it is thought that *C. rosea* may be a dominant species involved in domestic potato dry rot disease.

In the pathogenicity test, traces of strain invasion were observed in *B. ranunculi* and *H. nigrescens.* There are no reports on the involvement of *B. ranunculi* and *H. nigrescens* in dry rot disease. In particular, *H. nigrescens* is known to be non-pathogenic due to soil by-products, and in this experiment, it appeared to have been invaded through potato sclerosis. This suggests that *B. ranunculi* and *H. nigrescens* exhibited parasitic behavior, using potato sclera as nutrients in through wounds *in vivo*, and indicates that they are not directly related to the development of dry rot disease.

Declarations

Author contribution

NS Kim: Investigation, Data Curation. SJ Hong: Supervision, Validation, Writing, Review & Editing. HS Won: Review. BS Kim: Conceptualization, Methodology. SH Gwon: Investigation, Data Curation, Writing & Review.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Figures

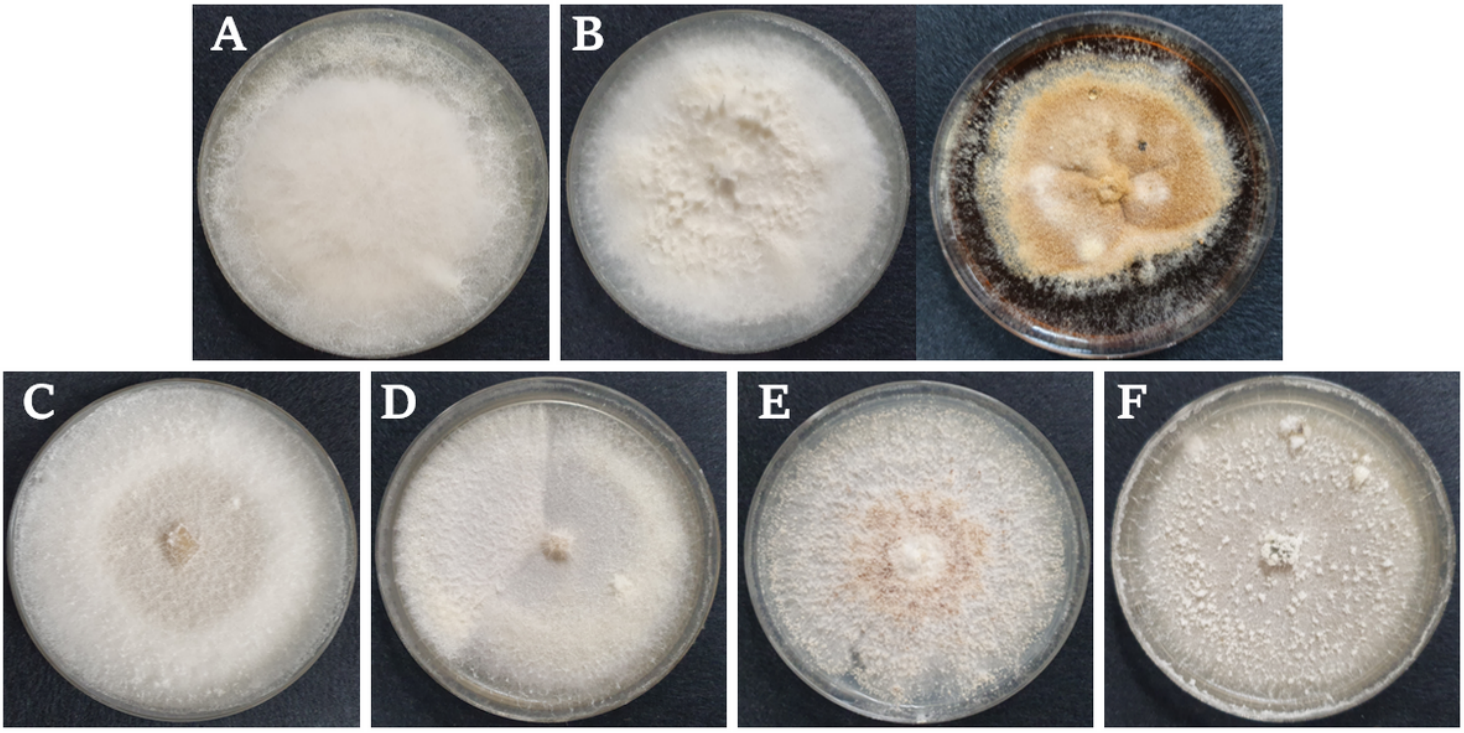

Figure 1

Colony morphologies of the isolated fungal species on water agar medium incubated at 25℃ for 3 days from potatoes showing symptoms of dry rot disease on tubers stored in cold storages. A, Beckjak; B, Dano; C, Doobak ; D, Jayoung; E, Superior; F, Wangsan.

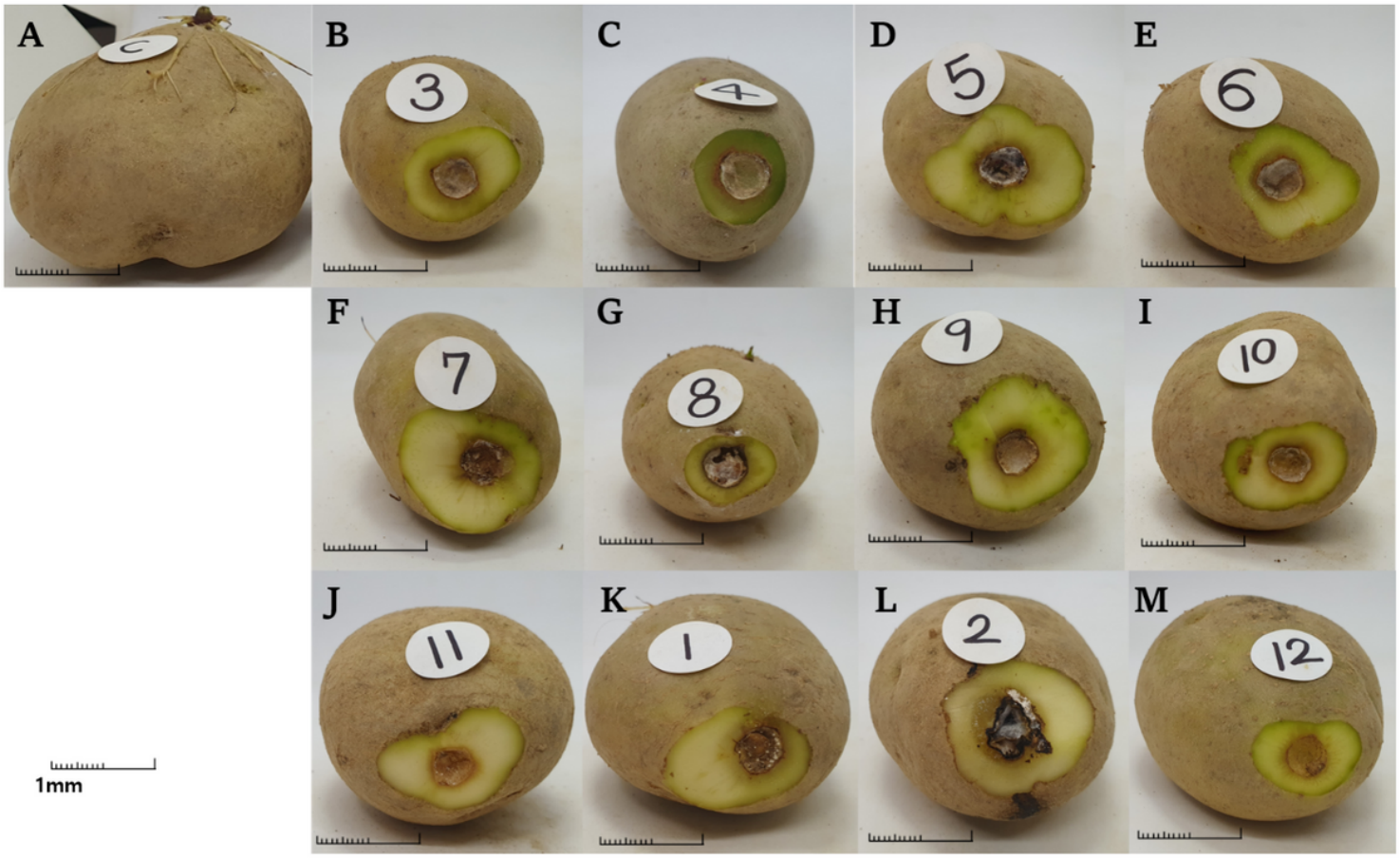

Figure 2

Disease symptoms of infected areas on potato tubers 7 weeks after the artificial inoculation of 12 fungal species incubated at 15~20℃ and 99% RH. A, Control, B; *Fusarium boothii*; C, *Fusarium circinatum*; D, *Fusarium citricola*; E, *Fusarium foetens*; F, *Fusarium iranicum*; G, *Fusarium longifundum,* H, *Fusarium oxysporum,* I, *Fusarium pseudoanthophilum,* J, *Fusarium solani, K*, *Botryotinia ranunculi*; L, *Clonostachys rosea*; M, *Humicola nigrescens.*

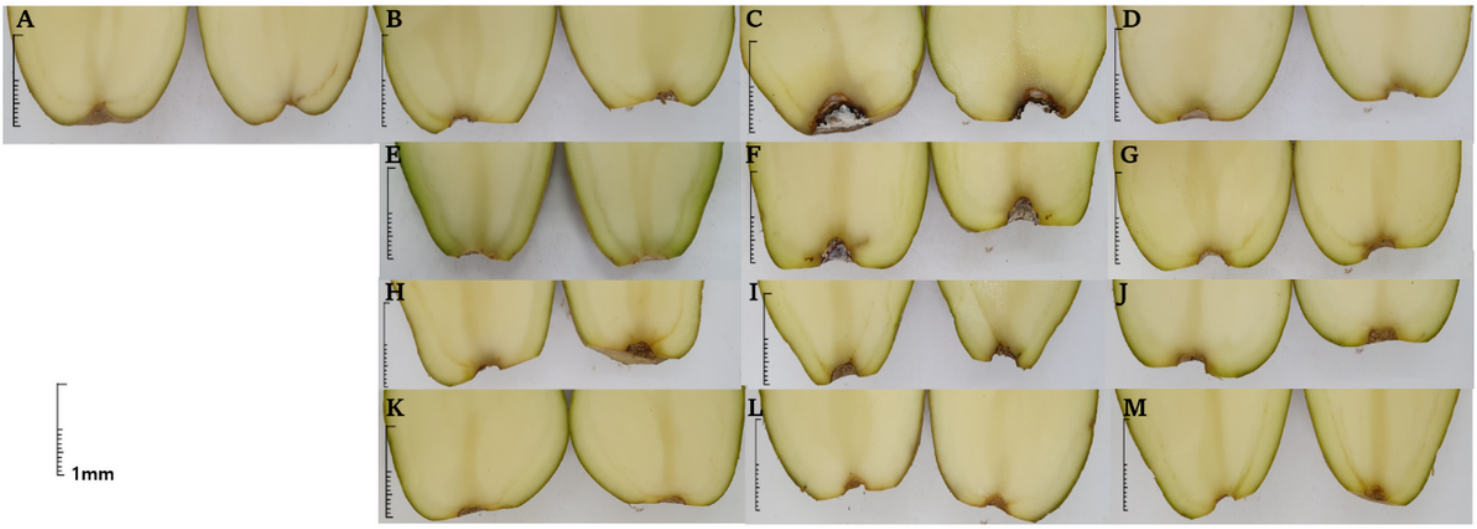

Figure 3

Appearance of the longitudinal sections of potato tubers 7 weeks after the artificial inoculation of 12 fungal species incubated at 15~20℃ and 99% RH. A, Control, B; *Fusarium boothii*; C, *Fusarium circinatum*; D, *Fusarium citricola*; E, *Fusarium foetens*; F, *Fusarium iranicum*; G, *Fusarium longifundum,* H, *Fusarium oxysporum,* I, *Fusarium pseudoanthophilum,* J, *Fusarium solani, K*, *Botryotinia ranunculi*; L, *Clonostachys rosea*; M, *Humicola nigrescens.*

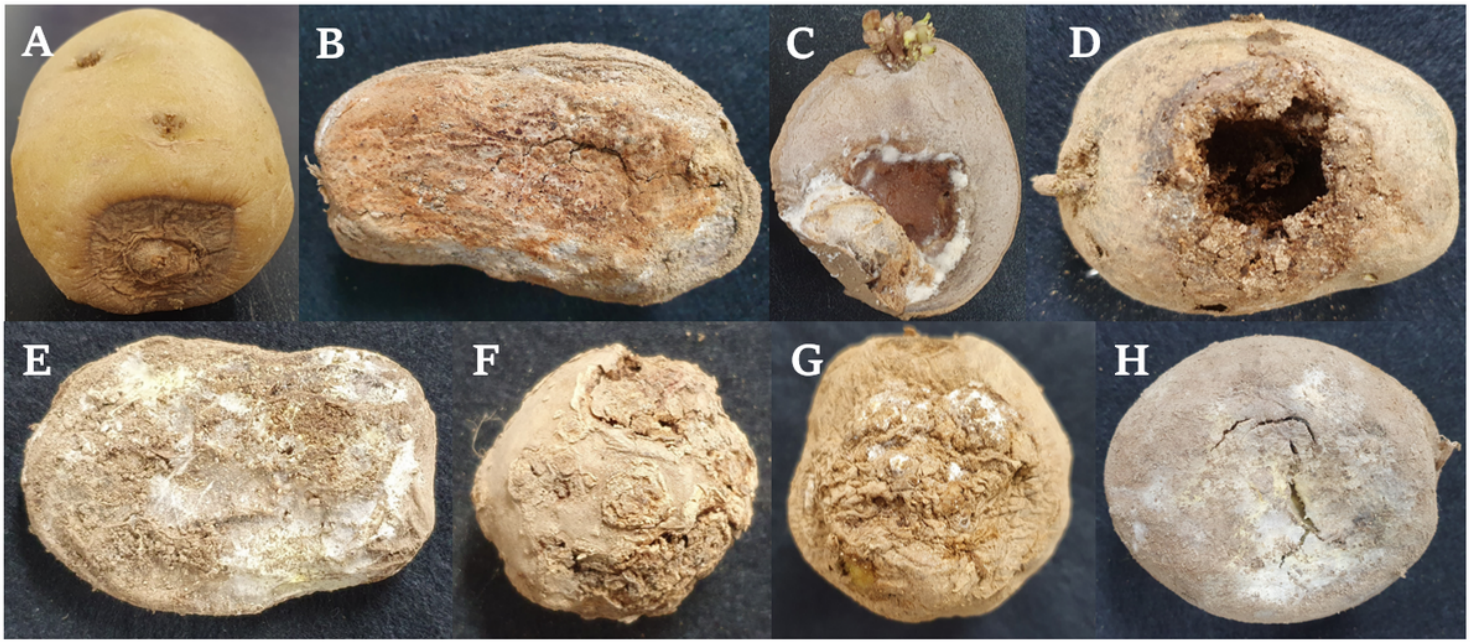

Figure 4

Potato tubers with symptoms of dry rot disease under common storage conditions (2021-2022). A; Atlantic, B; Beckjak, C; Chubaek, D; Dano, E; Doobak, F; Jayoung, G; Superior, H; Wangsan.

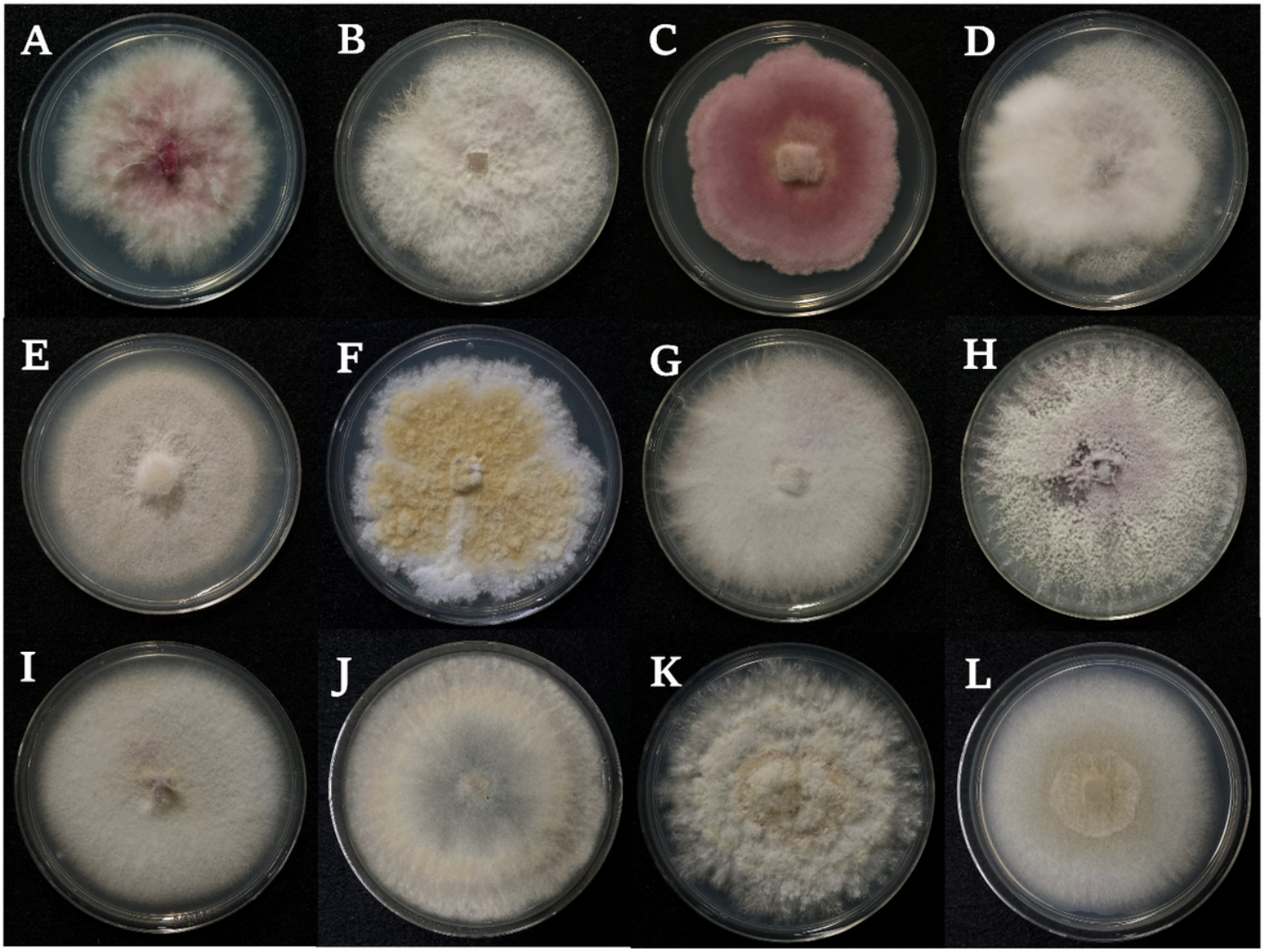

Figure 5

Colony morphology of the twelve isolated fungal species on potato dextrose agar medium incubated at 20°C for 7 days from potato tubers infected dry rot in this study. A, *Fusarium boothii*; B, *Fusarium circinatum*; C, *Fusarium citricola*; D, *Fusarium foetens*; E, *Fusarium iranicum*; F, *Fusarium longifundum,* G, *Fusarium oxysporum,* H, *Fusarium pseudoanthophilum,* I, *Fusarium solani,* J, *Botryotinia ranunculi*; K, *Clonostachys rosea*; L, *Humicola nigrescens*.

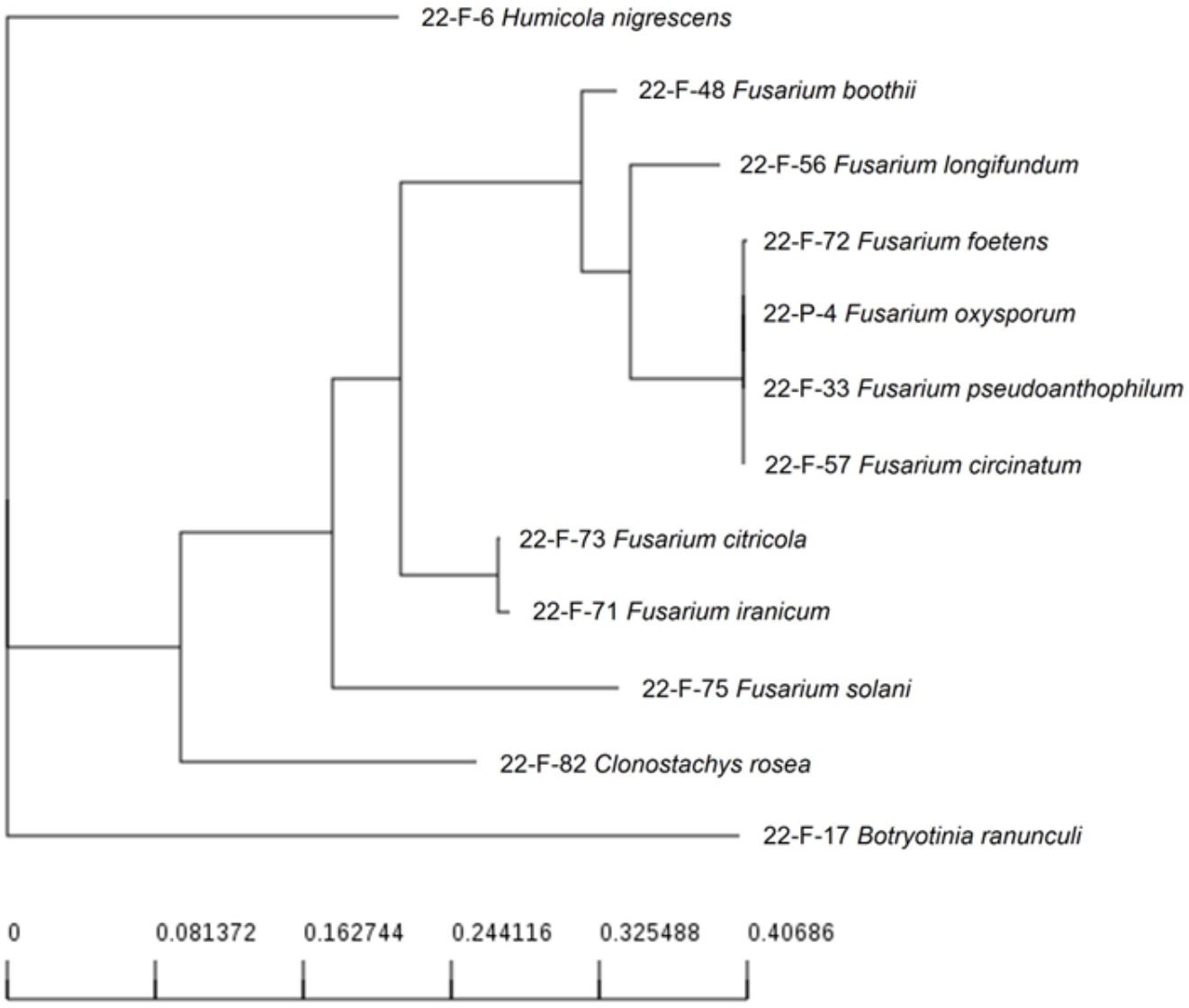

Figure 6

Phylogenetic relationship between *Fusarium spp.* and fungi isolated from potatoes from 76 fungi of 93 tubers showing symptoms of dry rot disease based on ITS region gene sequence analysis.