Phylogenetic analysis of *Microdochium* spp. associated with turfgrass and their pathogenicity in cereals

Filiz Ünal [1]

[1] Department of Plant Protection, Faculty of Agriculture, Eskişehir Osmangazi University, Eskişehir, Türkiye
Corresponding Author:
Filiz Ünal [1]
Eskişehir Osmangazi University Faculty of Agriculture, Ali Numan Kıraç Campus, Ziraat Street, Kütahya Road, 26160 Eskişehir/Türkiye
Email address: filiz.unal@ogu.edu.tr

Abstract

Turfgrass is frequently used today in the arrangement and aesthetic beautification of grounds in parks, gardens, median strips, recreation and sports areas. In this study, surveys were conduct in turfgrass areas in the three provinces of Türkiye. As a result of isolations from the collected samples, 44 *Microdochium* isolates obtained belonging to 5 different species including *M. bolleyi, M. majus, M. nivale, M. paspali* and *M. sorghi* ~~which have~~with different virulences. Identification of the isolates were performed by rDNA-ITS sequence analyzes. According to the results of pathogenicity tests~~ results~~, the most virulent species was *M. nivale* M62 with a disease severity value of 91.93%. This was followed by *M. bolleyi* M1584 and *M. majus* M63, with disease severity values of 91.12% and 91.08%, respectively. The virulence of *M. bolleyi* isolates varied among the species. Only 13 of the 31 *M. bolleyi* species were found to be virulent in turfgrass.~~,~~ *M. paspali* was less virulent than the others in *Poa pratensis*. The most virulent isolate of each *Microdochium* species was tested on 4 different cereal varieties. *M. sorghi* and *M. paspali* had low virulence values in barley and oat than the other *Microdochium* species, while the other three species showed high virulence in turfgrass, wheat and barley, other than oat. ~~In t~~The ~~n~~Neighboor-~~j~~Joining phylogenetic ~~neighboor joining~~ tree ~~belonging to~~constructing of the 44 *Microdochium* isolates clearly demonstrated that the isolates were grouped into five distinct clusters. *M. nivale and M. majus* were considered as genetically close isolates.

Key words: barley; identification; oat; phylogeny; survey; wheat

Introduction

Turfgrass areas, ~~whose~~ which cultivation area increases day by day, are the center of visual attention of people due to both their social and aesthetic appearance. In recent years, the interest in golf has led to an increase in grass areas requiring intensive maintenance. *Microdochium* genus

Formatted: Centered
Formatted: Justified
Formatted: Justified
Formatted: Justified

includes many species ~~have~~ are highly pathogenic, weakly pathogenic, endophyte, saprophyt~~ic~~ on cereals and grasses (Kirk and Deacon, 1987; Hernández-Restrepo et al., 2016; Demirci and Dane, 2003; Hong et al., 2008; Lench et al., 2014; Li et al., 2019; Gagkaeva et al., 2020; Huang et al., 2020). Pathogenic *Microdochium* spp. caused severe damage especially on wheat and barley. The fungi that cause the disease called "snow mold" or "*Microdochium* patch" on wheat, especially in cool climate conditions, are *Microdochium nivale* and *Microdochium majus*. These fungi cause economic losses in wheat and barley (Tronsmo and Matsumoto, 2001; Abdallah-Nekache et al., 2019; Mao et al., 2023). These two agents also cause head blight, which is frequently observed in wheat (Kammoun et al., 2009; Pancaldi et al., 2010; Hayashi et al., 2014). In a study conducted in Morocco, it was reported that wheat grains were highly contaminated with *M. majus* (Saoudi et al., 2019). Studies conducted in various regions of the world have shown that *Microdochium bolleyi* causes crown and root rots in grasses (Braun, 1995; Hong et al., 2008). There are studies reporting that *M. bolleyi* causes root and crown rot in wheat and barley as well as grass, as well as studies reporting that it is not a pathogen (Hannukkala and Koponen, 1987; Kendis et al., 1992; Lench et al., 2014; Li et al., 2019). For this reason, there are conflicting results regarding the hosts in which *M. bolleyi* causes disease. Another *Microdochium* species, *M. sorghi*, causes zonate leaf spots and rot on ~~*sorghum*~~ *Sorghum* spp. and other *Poaceae* (Von Arx, 1987; Braun, 1995), while *Microdochium paspali* causes seaside paspalum disease on *Paspalum vaginatum* (Zhang et al., 2015). It has been reported that *M. nivale* causes basal rot, crown rot, dryland root rot in dryland wheat fields in Türkiye, and also causes color change in seedlings or necrotic lesions in internode tissues in winter wheat (Demirci and Dane, 2003; Tunalı et al., 2008).

The rDNA region of microorganisms has been used frequently and successfully in molecular diagnosis, taxonomy studies and genetic diversity studies since the early 1980s. One of the reasons for this is the high copy number in the genome for DNA required for successful amplification, and also because the rDNA region consists of highly conserved regions that enable ~~the~~ design of general primers such as the ITS (White et al., 1990). This region is the most comprehensively sequenced and studied region in fungi (Schoch et al., 2009), which has enabled the creation of an enormous data pool (O'Brien et al., 2005), in addition to rDNA sequences such as 18S or 28S (Hernández-Restrepo et al., ~~others,~~ 2016; Gao et al., 2018), βtubulin (Myllys et al., 2002), RNA-polymerase binding proteins 1, 2 (Schoch et al., 2009), elongation factor-1α (Schoch et al., 2009) have been used frequently in fungi. The regions also used successfully in molecular phylogeny. In recent years, genetic differences between species within the *Microdochium* genus have been revealed using different gene regions (Hernández-Restrepo et al., 2016 ; Zhang et al., 2017 ; Crous et al., 2018 , 2019 ; Marin-Felix et al., 2019 ; Gao et al., 2019).

Microdochium species and their metabolites have also been reported to be used in the treatment of some diseases in plants and human~~s~~. For example; bioactive compounds of *Microdochium* species can be used succesfully against *Verticillium dahliae*, which causes plant vascular wilt (Berg et al., 2005). Cyclosporine A~~,~~ ; a metabolite is used to control ~~on~~ animal and human diseases, has been isolated from *Microdochium nivale* (Bhosale et al., 2011) and *M. phragmitis* extracts have been found to be cytotoxic in tumor cell lines in human~~s~~ (Santiago et al., 2012). Therefore, the

Formatted: Font: Italic, Complex Script Font: Italic

Formatted: Font: Italic, Complex Script Font: Italic

possibilities of using *Microdochium* species in biotechnology should be investigated in details in the future.

The aim of this study is to identify *Microdochium* species isolated from turfgrass roots, to determine the pathogenicity of the obtained species in turfgrass, wheat, barley and oats, and to reveal the genetic differences between species by phylogenetic analysis of the DNA sequences of the isolates.

Materials & Methods

Sample collection and isolations

Surveys were conducted turfgrass areas in Eskişehir, Ankara and Kocaeli provinces of Türkiye and turfgrass samples were collected. In the isolations from the plants, tissue pieces of the diseased root and crown ~~root~~ were dried on sterile blotting paper after 1 minute surface disinfection in 1% sodium hypochlorite. Then, it was placed on potato dextrose agar~~.~~ (Difco, USA). After 3-4 days, the hyphae tips of Microdochium-like fungi were removed with a sterile loop and transferred to PDA, and then to water agar (Merck, Germany) for single spor isolation (Less et al., 1995).

Pathogenicity tests

Before pot experiments, preliminary pathogenicity tests were performed in Petri dishes to eliminate non-pathogenic isolates. ~~In~~ Preliminary pathogenicity assays were performed according to Ichielevich-Auster et al. (1985) using susceptible turfgrass (*Poa pratensis*). As a result of the assays, those showing symptoms such as light discoloration in the hypocotyl and roots, rot, and root loss were selected for pot trials. Inoculum obtained by wrapping the fungi into rye seeds was used in pot analyses. Then, this inoculum was mixed at 5% into the prepared mixture (soil, sand and manure) in a 2:1:1 ratio. Each application was carried out in three repetitions with square pots of 12cm x 12cm (Smiley, 2019). No inoculum was added to the control pots. The pots were covered with nylon bags and left to incubate for 3 days. After incubation was completed, thirty *P. pratensis* seeds were planted in each pot and covered with soil. After planting, each pot was watered with approximately 20 ml of sterile water. After 4 weeks, grass plants were examined and evaluated using a 0–3 scale (Cong et al., 2016). Finally, disease severity values were calculated using the Townsend and Heuberger ~~Town. and Heuber.~~ formula (Townsend and Heuberger, 1943). At the end of the study, reisolation of fungi from plants was carried out. In consequence of the pathogenicity tests, isolates with disease severity values between 80 % - 100 % were considered highly virulent, isolates with disease severity values between 70 % - 80 % were considered moderately virulent, and isolates with disease severity values at 70% and below were considered as low virulent.

Data were subjected to analysis of variance (ANOVA) with Statistical version 12. Significance of mean differences in disease severity were determined using the Tukey's test with $p = 0.05$ as a significance threshold.

Pathogenicity studies of *Microdochium* species in cereals

Formatted: Justified

Formatted: Font: Not Italic, Complex Script Font: Not Italic

Formatted: Font: Italic, Complex Script Font: Italic

In the study, the most virulent isolate isolated in each *Microdochium* species were selected and their virulences were investigated in selected turfgrass, wheat, barley and oat varieties. Tall fescue turfgrass (*Festuca arundinacea* Schreb), Tarm-92 barley (*Hordeum vulgare* L.), Kızıltan-91 wheat (*Triticum aestivum* L.) and Seydişehir oat (*Avena sativa* L.) varieties were used in the trials. The experiments were carried out in greenhouse conditions with pot trials in 3 replicates. Inoculum preparation and application of inoculum to plant roots and evaluations were carried out as in pathogenicity tests. In pathogenicity tests were conducted on the cereals, cereals had disease severity values between 80 % - 100 % were considered susceptible to isolate, cereals with disease severity values between 70 % - 80 % were considered moderately susceptible to isolate, cereals with disease severity values between 70% - 50% were considered moderately resistant, and those below 50% were considered resistant.

Data were subjected to analysis of variance (ANOVA) with Statistical version 12. Significance of mean differences in disease severity were determined using the Tukey's test with $p = 0.05$ as a significance threshold. The data obtained in the study was analyzed according to a completely random design.

Molecular identification and phylogenetic evaluation

In molecular studies, ITS regions of rDNA of *Microdochium* isolates were amplified using the universal primer pairs, ITS 4 and 1~~,~~ (White et al., 1990). DNAs were isolated by using Blood and Tissue Kit (QIAGEN), according to the instructions for use. Each PCR reaction contained 12 µL Taq DNA Polymerase 2x Master Mix, 1 µL of each primer, 3 µL of template DNA. The PCR reaction mix was adjusted to a final volume of 25 µL with ultrapure water. PCR amplifications were carried out on the ABI Veriti thermal cycler. ~~It was used the~~ The following cycling protocols were used (Hong et al., 2008): The thermal cycler parameters were programmed for 1 cycle of denaturation at 95℃ for 4 min, 35 cycles of denaturation at 95℃ for 1 min, annealing at 58℃ for 1 min, extension at 72℃ for 1 min, and a final extension at 72℃ for 7 min. PCR products were analyzed by electrophoresis on a 2% agarose gel prepared with Pronosafe (Conda, Spain) DNA dye and visualized under UV transilluminator. Sequence analyzes were performed by BM Lab (Ankara, Türkiye). Then Blast analyzes of isolates were made using these sequences on NCBI (http://www.ncbi.nlm.nih.gov) to find the closest matches. Sequences were edited and aligned by the CLUSTAL W method (Thompson et al., 1994). The phylogenetic tree was constructed using the ITS sequences of the fungi using the neighbor-joining method ~~using~~ by MEGA ver 7.0 software (Kumar et al., 2016), sequence distance was calculated with Kimura 2-parameter model (Kimura, 1980). Bootstrap analysis was performed with 1-000 replications to determine the support for each clade.

Results

A total of 153 grass samples were collected from parks, gardens, recreation areas, stadiums and median strips in Ankara, Eskişehir and Kocaeli provinces, where yellowing, wilting, blight, necrosis and rotting of roots, and rings and patches on the ground were observed. As a result of the isolations made from the roots and crowns ~~roots~~ of the samples, 44 fungal isolates belonging to the *Microdochium* genus were obtained. As a result of rDNA-ITS sequencing and BLAST analysis,

Formatted: Justified

31 of the 44 *Microdochium* isolates obtained were identified as *M. bolleyi*, 7 as *M. nivale*, 3 as *M. paspali*, 2 as *M. majus* and 1 as *M. sorghi*.

As a result of preliminary pathogenicity tests performed in Petri dishes using the turfgrass sensitive variety *Poa pratensis,* which is sensitive to *Fusarium* species, 18 species of *M. bolleyi* were evaluated as non-pathogenic and eliminated because they did not show any disease symptoms in the roots and hypocotyls. The other 13 *M. bolleyi* species caused necrosis and decays on hypocotyls. All the other *Microdochium* species subjected to preliminary pathogenicity ~~testing~~ tests caused necrosis and decays on hypocotyls. The other *Microdochium* isolates caused hypocotyl necrosis and decays in the preliminary pathogenicity tests. Pathogenicity studies were conducted in pots with 26 *Microdochium* species, which were evaluated as ~~pathogens~~ virulent in preliminary pathogenicity tests (Table 1). As a result of pathogenicity studies conducted under greenhouse conditions, the most virulent isolate was determined to be *M. nivale* M62 with a disease severity value of 91.93 %. This was followed by *M. bolleyi* M1584 isolate with a disease severity of 91.12 %. The lowest virulence value among *M. bolleyi* isolates was *M. bolleyi* M732 isolated from Ankara with 70.04% disease severity and was evaluated as moderately virulent. The virulence values of *M. bolleyi* isolates isolated from turfgrass areas varied between moderately and highly virulent (Table 1).

When *M. nivale* isolates were evaluated, 6 isolates except *M. nivale* M171 were determined to be highly virulent with disease severity values between 85.04 % and 91.93 %. Among *M. paspali* species, the most virulent isolate was *M. paspali* M874 with a disease severity of 87.66 %. The disease severity in the other two *M. paspali* species was calculated as 72.12 % and 77.74 % and was considered as moderately virulent. All *M. majus* and *M. sorghi* isolates were found highly ~~virülent~~ virulent (Table 1, Figure 1). When pathogenic and non-pathogenic isolates are evaluated together, the most *Microdochium* isolates were isolated from Kocaeli province, while the most pathogenic isolates were isolated from Eskişehir province. In addtion, higly pathogenic *M. bolleyi* isolates were obtained from Kocaeli province, which is mostly coastal and has a more temperate climate than other provinces. More isolates of the *M. nivale* species were obtained in Ankara and Eskişehir, which have a continental climate, and it was determined that *M. nivale* species obtained from these two provinces were more virulent in turfgrass (Table 1).

By selecting the most virulent isolates of 5 species isolated from turfgrasses, their virulences were investigated in Tall fescue turfgrass (*Festuca arundinacea* Schreb), Tarm-92 barley (*Hordeum vulgare* L.), Kızıltan-91 wheat (*Triticum aestivum* L.) and Seydişehir oat (*Avena sativa* L.) varieties, which are widely used in Türkiye (Table 2).

F. arundinacea was found to be susceptible to all 5 *Microdochium* species, showing disease severity values between 79.46 % - 92.96 %. The most virulent species in *F. arundinacea* was *M. bolleyi*, which showed a disease severity value of 92.96 %. This was followed by *M. majus* with a value of 92.18%. *M. paspali* was found to cause less severe disease (79.46 %) in *F. arundinacea* compared to the other 4 *Microdochium* species. *T. aestivum* was found to be susceptible to all *Microdochium* species, showing disease severity of 83.29 %-91.52 %. The species that caused the most severe disease in *T. aestivum* was *Microdochium majus*. *Hordeum vulgare* was found to be sensitive to *M. bolleyi, M. majus* and *M. nivale,* while it was found to be moderately sensitive to

Formatted: Justified

M. sorghi and *M. paspali* compared to the other three species, with disease severity values of 69.75% and 74.67%, respectively. While *Avena sativa* showed a moderately resistant reaction to *M. paspali* with a disease severity value of 63.29%, it showed a moderately sensitive reaction to the other 4 species (Table 2, Figure 3).

Phylogenetic tree ~~constructed~~ with the *Microdochium* isolates isolated from turfgrass was constructed by bootstrap neighbor-joining analysis of nucleotide sequences to evaluate genetic differences among isolates belonging to 5 *Microdochium* species. The ~~phylogenetic neighboor~~Neighboor-~~joining~~ Joining phylogenetic tree belonging to *Microdochium* isolates clearly demonstrated that the isolates were grouped into 5 distinct clusters (Figure 1). It was observed that *M. nivale* and *M. majus* species formed their own small groups within the same group on the tree. This situation showed that these two species were genetically closely related species. Isolates of ~~belonged~~ the other 4 species were grouped within themselves and formed groups in different places on the tree than the groups of other species (Figure 4).

Discussion

The aim of this study was to identify *Microdochium* species isolated from turfgrass fields, to investigate the pathogenicity of the identified species on turfgrass, wheat, barley and oat, and to investigate the genetic variation among all isolates. As a result of the surveys carried out in the spring, it was determined that the dominant species in Eskişehir, Ankara and Kocaeli ~~Provinces~~ provinces was *M. bolleyi*. This was followed by *M. nivale*. *M. bolleyi* was generally isolated from the seaside province of Kocaeli, and the virulence of ~~*Microdochium*~~ *M. bolleyi* species isolated from this province was higher than the others. This suggests that *M. bolleyi* is better adapted to regions with humid and temperate climates. *M. nivale, M. majus* and *M. sorghi* ~~are~~ were more isolated from Ankara and Eskişehir, which have a continental and relatively colder climate. It is known that *Microdochium nivale* survives even at low temperatures and causes serious infections, especially in grass and wheat (Tronsmo et al., 2001). In a study conducted in China, it was reported that *M. paspalum* caused leaf necrosis in the grass species *Paspalum vaginatum* on golf courses in late winter and early spring.

In this study, while all of the *M. nivale*, *M. paspali*, *M. majus* and *M. sorgi* species isolated from grass areas were found to be pathogenic~~s~~ in turfgrass, only 13 of the 31 *Microdochium bolleyi* species isolated were found to be pathogen~~s~~ic in turfgrass. Pathogenicity values of *M. bolleyi* isolates also varied.

While it was stated in some previous studies that *Microdochium bolleyi* was non-pathogenic, endophytic (Maydan et al., 2010) and weak parasite (Kirk et al., 1987) agent, in some studies, it was found to be a pathogen, especially in turfgrasses, in recent years. In a study conducted in Finland, it was reported that *M. bolleyi* is a fungus commonly found on barley and wheat roots. Additionally, *M. bolleyi* is reported to be isolated from diseased roots, often together with other fungi that cause disease in cereals. In the same study, it was reported that the agent also colonized other plants other than oats and cereals, although not as much as wheat and barley. Hemens et al., (1992) stated that *Microdochium bolleyi* developes within roots and coleoptiles of barley either

Formatted: Justified

Formatted: Justified

Formatted: Justified

leading to a damage of the tissue or to a symptomless infection. They indicated that the hyphae of the agent were seen in both, roots and coleoptiles in dead cells only. On the other hand, It was reported that *M. bolleyi* causing root rot on naked barley in China (Li et al., 2019). It has even been reported to cause damping-off on wheat (Lenc et al., 2013). In this study, although the majority of *M. bolleyi* Türkiye isolates ~~isolated from Türkiye~~ were determined to be non-pathogenic, pathogenic isolates were determined to be highly virulent, especially in grass, wheat and barley. Wheat, barley and grass in particular were found to be susceptible to virulent strains of the agent. Similarly, it has been reported that *M. bollyei* causes basal rot and causes significant damage to turfgrasses on golf courses in Korea (Hong et al., 2008).

In this study, All *Microdochium majus* and *M. nivale* isolates ~~isolated~~ from turfgrass were higly pathogenic on turfgrass and the other cereals. These two species were isolated especially from terrestrial and cold areas and their disease severity values were found to be quite high. It was stated that *M. nivale* species are common plant pathogens in cool and temperate regions of the northern hemisphere (Lees et al., 1995; Tronsmo et al., 2001; Waalwijk et al., 2003; ~~Kammoun et al., 2009;~~ Hong et al., 2008; Kammoun et al., 2009). Although some studies indicated that *M. majus* are relatively less pathogenic than *M. nivale* on grasses (Holmes, 1976; Hofgaard et al., 2006~~; Holmes, 1976~~), in this study, *M. majus* caused disease severity similar to *M. nivale*. *M. majus* caused brown foot rot in wheat (Ma et al., 2023). Both species cause significant damage to cereals (Diamond et al., 1995). In this study all. *M. paspali* species were found to be moderately pathogenic in turfgrass. *M. paspali* species were isolated from all 3 provinces and the most virulent *M. paspali* strain was isolated from Eskişehir, Türkiye. In a study conducted in China, it was reported that *M. paspali* caused serious damage on golf courses in regions with cold climate periods (Zhang et al., 2015). *M. sorghi* causes zonate leaf spots and decay on *Sorghum* species and other *Poaceae* (Von Arx, 1987; Braun, 1995). In this study, while *M. sorghi* was highly virulent in turfgrass and wheat, it was found to be moderately virulent in barley and oats. In the future, studies should be conducted to reveal the virulence status of *Microdochium* species isolated from different geographical regions and climatic conditions in ~~*Gramineae*~~*Poaceae*.

In recent years, some studies have been carried out to determine the differences between species within *Microdochium* and to clarify their taxonomy and some species have been reclassified based on molecular analyses (Glynn et al., 2005; Jewell and Hsiang, 2013; Hernández-Restrepo et al., 2016). For example, phylogenetic analysis of the translation elongation factor 1-alpha gene (TEF1) showed that the isolates previously described as varieties of *Microdochium nivale* shown a distinct heterogeneity between isolates and these isolates were generated as two separate species, *M. majus* and *M. nivale* (Glynn et al., 2005). In this study, with different *Microdochium* species isolated from turfgrass, a Neighbour-Joining phylogenetic tree was constructed with 1000 bootstrap replicates. The phylogenetic tree was constructed based on the internal transcribed spacer (ITS) genetic region of 44 isolates that including different *Microdochium* isolates. ITS is the most commonly used in phylogenetic studies of fungal isolates, followed by β-tubulin, EF-1α and RPB2 (Hibbett et al., 2016). Genetic differences between species in five different Microdochium species were detected on the tree. This result agrees with previous studies by Glynn et al. (2005) and Jewell and Hsiang (2013). Our results do support the proposition by Glynn et al. (2005) that *M. nivale* and

Formatted: Font: Italic, Complex Script Font: Italic

M. majus should be considered as two separate species. However, Jewell and Hsiang (2013) were not able to differentiate between these two fungal species using the ITS sequences. Similarly, in our study the ITS sequences were the least informative of all. The multi-copy nature of the ITS sequence makes it easy to amplify from low-quality DNA, however, it also limits the ability of this genomic region to distinguish between interspecific and intraspecific variation (~~Hibbett et al., 2016;~~ James et al., 2006; O'Donnell et al., 2015; Hibbett et al., 2016).

M. bolleyii, M. paspali and *M. sorghi* isolates were grouped among themselves and settled in different parts of the tree, but *M. nivale* and *M. majus* species formed 2 different small groups within a single large group, showing that they were two different species close to each other. It was observed that *M. nivale* species showed minor differences within themselves.

Conclusions

In conclusion, the results obtained the genotypic and pathogenic differences detected between isolates from this study support the species-based differences of *Microdochium* genus members. Future investigations of the relationships between host diversity and genetic diversity of isolates and the effects of different geographical regions and climatic conditions on genetic diversity will bring interesting results. Further studies may reveal ~~that~~ new *Microdochium* isolates, their virulence in different hosts and management.

References

Abdallah-Nekache, N, Laraba I, Ducos C, Barreau C, Bouznad Z, Boureghda H. 2019. Occurrence of *Fusarium* head blight and *Fusarium* crown rot in Algerian wheat: Identification of associated species and assessment of aggressiveness. *Eur. J. Plant Pathol.* **154**: 499–512. https://doi.org/10.1007/s10658-019-01673-7.

Berg G, Zachow C, Lottmann J, Götz M, Costa R, Smalla K. 2005. Impact of Plant Species and Site on Rhizosphere-Associated Fungi Antagonistic to *Verticillium dahliae. Kleb. Appl. Environ. Microbiol.* **71**: 4203–4213. https://doi.org/10.1128/AEM.71.8.4203-4213.2005.

Bhosale SH, Patil KB, Parameswaran PS, Naik CG, Jagtap TG. 2011. Active pharmaceutical ingredient (api) from an estuarine fungus, *Microdochium nivale* (Fr.). *J. Environ. Biol.* **32**: 653–658. http://drs.nio.org/drs/handle/2264/3917.

Braun UA. 1995. Monograph of *Cercosporella, Ramularia*, and *Allied* Genera (Phytopathogenic Hyphomycetes); Ihw-Verlag: Eching, Germany, Volume 1.

Cong LL, Sun Y, Kang JM, Li MN, Long RC, Zhang TJ, Yang QC. 2016. First report of root rot disease caused by *Fusarium proliferatum* on alfalfa in China. *Plant Dis.* **100:** 2526. https://doi.org/10.1094/PDIS-04-16-0505-PDN

Crous PW, Schumacher RK, Wingfield MJ, Akulov A, Bulgakov TS, Carnegie AJ, Jurjević Ž, Decock C, Denman S, Lombard L, et al. 2018. New and interesting fungi. 1. *Fungal Syst. Evol.* **1**: 169–215. https://doi.org/10.3114/fuse.2018.01.08.

Demirci E, Dane E. 2003. Identification and pathogenicity of *Fusarium* spp. from stem bases of winter wheat in Erzurum, Turkey. *Phytoparasitica* **31**: 170–173. https://doi.org/10.1007/BF02980787.

Formatted: Justified

Formatted: Justified

Formatted: Justified

Diamond H, Cooke BM, Dunne B. 1995. Information note: *Microdochium* leaf blotch of oats—a new foliar disease caused by *Microdochium nivale* var. *nivale* in Ireland. *Plant Var. Seeds* **8**: 171–174.

https://scholar.google.com/scholar_lookup?title=Information+note:+Microdochium+leaf+blotch+of+oats%E2%80%94a+new+foliar+disease+caused+by+Microdochium+nivale+var.+nivale+in+Ireland&author=Diamond,+H.&author=Cooke,+B.M.&author=Dunne,+B.&publication_year=1995&journal=Plant+Var.+Seeds&volume=8&pages=171%E2%80%93174.

Gagkaeva TY, Orina AS, Gavrilova OP, Gogina NN. 2020. Evidence of *Microdochium* Fungi Associated with Cereal Grains in Russia. *Microorganisms* **8**: 340. https://doi: 10.3390/microorganisms8030340.

Gao Q, Jin K, Ying S, Zhang Y, Xiao G, Shang Y, Duan Z, Hu X, Xie X, Zhou G. 2011. Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. *Plos Genet.* **5**: 122. https://doi.org/10.1371/journal.pgen.1001264.

Glynn NC, Hare MC, Parry DW, Edwards SG. 2005. Phylogenetic analysis of EF-1 alpha gene sequences from isolates of *Microdochium nivale* leads to elevation of varieties majus and nivale to species status. *Mycol. Res.* **109**: 872–880. https://doi.org/10.1017/S0953756205003370.

Hannukkala A, Koponen H. 1988. *Microdochium bolleyi*, a common inhabitant of barley and wheat roots in Finland. *Karstenia* **27**: 31-36.

Hayashi Y, Kozawa T, Aiuchi D, Koike M, Akino S, Kondo N. 2014. Population genetic structure of *Microdochium majus* and *Microdochium nivale* associated with *Fusarium* head blight of wheat in Hokkaido, Japan. *Eur. J. Plant Pathol.* **140**: 787–795. https://doi.org/10.1007/s10658-014-0509-3.

Hemens E, Steiner U, Schnbeck F. 1992. Infektions-strukturen von *Microdochium bolleyi* an Wurzeln und Koleoptilen von Gerste. *Journal of Phytopathology* **136**: 57–66.

Hernández-Restrepo M, Groenewald JZ, Crous PW. 2016. Taxonomic and phylogenetic re-evaluation of *Microdochium*, *Monographella* and *Idriella*. Pers. *Mol. Phylogeny Evol. Fungi* **36**: 57-82. https://doi.org/10.3767/003158516X688676.

Hibbett D, Abarenkov K, Kõljalg U, Öpik M, Chai B, Cole J, Wang Q, Crous P, Robert V, Helgason T, et al. 2016. Sequence-based classification and identification of Fungi. *Mycologia* **108**: 1049–1068. https://doi.org/10.3852/16-130.

Hofgaard IS, Wanner LA, Hageskal G, Henriksen B, Klemsdal SS, Tronsmo AM. 2006. Isolates of *Microdochium nivale* and *M. majus* differentiated by pathogenicity on perennial ryegrass (*Lolium perenne* L.) and in vitro growth at low temperature. *J. Phytopathol.* **154:** 267–274. https://doi.org/10.1111/j.1439-0434.2006.01092.x

Holmes SJI 1976. Comparative study of the infection of perennial ryegrass by *Fusarium nivale* and *F. culmorum* in sterilized soil. *Ann. Appl. Biol.* **84**: 13–19.

Hong SK, Kim WG, Choi W, Lee SY. 2008. Identification of *Microdochium bolleyi* associated with basal rot of creeping bent grass in Korea. *Mycobiology* **36**: 77–80. http://dx.doi.org/10.4489/MYCO.2008.36.2.077.

Huang ST, Xia JW, Zhang XG, Sun WX, Li Z. 2020. Two new species of *Microdochium* from *Indocalamus longiauritus* in south-western China. *MycoKeys* **72**: 93–108. https://doi.org/10.3897/mycokeys.72.55445.

Ichielevich-Auster M, Sneh B, Koltin Y, Barash I. 1985. Suppression of damping-off caused by *Rhizoctonia* species by a non-pathogenic isolate of *R. solani*. *Phytopathology* **75**: 1080–1084. https://scholar.google.com/scholar_lookup?title=Suppression%20of%20dampingoff%20caused%20by%20Rhizoctonia%20species%20by%20a%20nonpathogenic%20isolate%20of%20R.%20solani&publication_year=1985&author=M%20IchielevichAuster&author=B%20Sneh&author=Y%20Koltin&author=I%20Barash.

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J. et al. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **443**: 818–822. https://www.nature.com/articles/nature05110.

Jewell LE 2013. Genetic and Pathogenic Differences between *Microdochium nivale* and *Microdochium majus*. Ph.D. Thesis, The University of Guelph, Guelph, ON, Canada. https://doi.org/10.1139/cjb-2012-0178.

Kammoun LG, Gargouri S, Hajlaoui MR, Marrakchi M. 2009. Occurrence and distribution of *Microdochium* and *Fusarium* species isolated from durum wheat in northern Tunisia and detection of mycotoxins in naturally infested grain. *J. Phytopathol.* **157**: 546–551. https://doi.org/10.1111/j.1439-0434.2008.01522.x.

Kimura MA 1980. simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**: 111–120. https://link.springer.com/article/10.1007/BF0173158142.

Kirk JJ, Deacon JW 1987. Control of the take-all fungus by *Microdochium bolleyi* and interactions involving *M. bolleyi*, *Phialophora graminicola* and *Periconia macrospinosa* on cereal roots. *Plant and Soil* **98**: 231–237. https://doi.org/doi:10.1007/BF02374826.

Kumar S, Stecher G, Tamura K. 2016. MEGA 7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lees A, Nicholson P, Rezanoor H, Parry D. 1995. Analysis of variation within *Microdochium nivale* from wheat: Evidence for a distinct sub-group. *Mycol. Res.* **99**:103–109. https://scholar.google.com/scholar_lookup?title=Suppression%20of%20dampingoff%20caused%20by%20Rhizoctonia%20species%20by%20a%20nonpathogenic%20isolate%20of%20R.%20solani&publication_year=1985&author=M%20IchielevichAuster&author=B%20Sneh&author=Y%20Koltin&author=I%20Barash.

Lenc L, Kwaśna H, Sadowski C, Grabowski A. 2014. Microbiota in wheat roots, rhizosphere and soil in crops grown in organic and other production systems. *J. Phytopathol.* **163***:* 245–263. https://doi.org/10.1111/jph.12313

Li XP, Li JH, Qi YH. 2019. Identification of the pathogens causing *Microdochium* root rot on naked barley. Acta Phytopathologica *Sinica* **49**: 705-710. https://doi.org/10.11686/cyxb2020270

Mao Y., Wu J, Song W, Zhao B, Zhao H, Cai Y, Wang JX, Zhou M, Duan Y. 2023. Occurrence and chemical control strategy of wheat brown foot rot caused by *Microdochium majus*. *Plant Dis. Jul* **24**. https://doi: 10.1094/PDIS-02-23-0392-RE.

Marin-Felix Y, Groenewald JZ, Cai L, Chen Q, Marincowitz S, Barnes I, Bensch K, Braun U, Camporesi E, Damm U. et al. 2017. Genera of Phytopathogenic Fungi: GOPHY 1. *Stud. Mycol.* **86**: 99–216. https://doi.org/10.1016/j.simyco.2017.04.002.

Myllys L, Stenroos S, Thell A. 2002. New genes for phylogenetic studies of lichenized fungi: Glyceraldehyde-3-phosphate dehydrogenase and betatubulin genes. *Lichenologist* **34**: 237–246. https://doi.org/10.1006/lich.2002.0390.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R. 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Appl. Environ. Microbiol.* **71**: 5544–5550. https://doi.org/10.1128/AEM.71.9.5544-5550.2005

O'Donnell K, Ward TJ, Robert VARG, Crous PW, Geiser DM, Kang S. 2015. DNA Sequence-Based Identification of *Fusarium*: Current Status and Future Directions. *Phytoparasitica* **43**: 583–595. https://doi.org/10.1007/s12600-015-0484-z.

Pancaldi D, Tonti S, Prodi A, Salomoni D, Dal Pra M, Nipoti P, Alberti I, Pisi A. 2010. Survey of the main causal agents of *Fusarium* head blight of durum wheat around Bologna, northern Italy. *Phytopathol. Mediterr.* **49**: 258–266. https://www.jstor.org/stable/26458600

Santiago IF, Alves TM, Rabello A, Junior PAS, Romanha AJ, Zani CL, Rosa CA Rosa LH. 2012. Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antarctic angiosperms *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. *Extremophiles* **16**: 95–103. https://doi.org/10.1007/s00792-011-0409-9.

Saoudi G, El Ghadraoui L, El Ghachtouli N. et al. 2019. Propagation of Wheat *Fusarium* wilt in Morocco. *Int J Sci Res.* **8**: 221–232. https://doi.org/10.21275/ART20195876

Schoch CL, Sung GH, López-Giráldez F, Townsend JP, Miadlikowska J. et al. 2009. Phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Systematic Biology* **58**:224–239. https://doi: 10.1093/sysbio/syp020.

Smiley RW 2019. Mechanized Method to inoculatedfield soil to evaluate *Fusarium* crown rot of wheat. *Plant Dis.* **103**: 2857-2864. https://doi.org/10.1094/PDIS-04-16-0505-PDN

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*. **22**: 4673–4680. https://doi.org/110.1093/nar/22.22.4673.

Townsend GK, Heuberger TW. 1943. Methods for estimating losses caused by diseases in fungicide experiments. *Plant Dis.* **27**: 340–343.

https://scholar.google.com/scholar_lookup?title=Methods+for+estimating+losses+caused+by+diseases+in+fungicide+experiments&author=Townsend,+G.K.&author=Hunburger,+T.W.&publication_year=1943&journal=Plant+Dis.&volume=27&pages=340%E2%80%93343.

Tronsmo AM, Hsiang T, Okuyama H, Nakajima TA. 2001. Low temperature diseases caused by *Microdochium nivale*. In Low Temperature Plant Microbe Interactions under Snow; Iriki, N., Gaudet, D.A., Tronsmo, A.M., Matsumoto, N., Yoshidaand, M., Nishimune, A., Eds.; Hokkaido

National Agricultural Experiment Station: Hokkaido, Japan, pp. 75–86. https://www.researchgate.net/publication/242176710

Tunali B, Nicol JM, Hodson D, Uçkun Z, Büyük O, Erdurmuş D, Bağci SA. 2008. Root and crown rot fungi associated with spring, facultative, and winter wheat in Turkey. *Plant Dis.* **92**: 1299–1306.

Von Arx JA. 1987. Plant pathogenic fungi. *Mycologia* **79**: 919–920. https://doi.org/10.1094/PDIS-92-9-1299.

Waalwijk C, Kastelein P, Vries ID, Kerényi Z, Van der Lee T, Hesselink T, Köhl J, Kema G. 2003. Major changes in *Fusarium* spp. in wheat in the Netherlands. *Eur. J. Plant Pathol.* **109**: 743–754. https://link.springer.com/article/10.1023/A:1026086510156.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In PCR Protocols; Elsevier: Amsterdam, The Netherlands, pp. 315–322.

Zhang W, Nan Z, Tian P, Hu M, Gao Z, Li M, Liu G. 2015. *Microdochium paspali*, a new species causing seashore paspalum disease in southern China. *Mycologia* **107**: 80–89. https://doi.org/10.3767/persoonia.2017.39.01.