Communication

The Effect of Dark Septate Endophytic Fungi on *Mahonia oiwakensis*

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Abstract: This is the first study to discuss the effects of dark septate endophytes (DSE) on the growth promotion and berberine concentration in *Mahonia oiwakensis*, whose extract (MOE) has been suggested to have potential therapeutic effects against human lung cancer. First, as per phylogenetic analysis, the strains were divided into four groups: CkDB2, CkDB5, MoAL2 and MoAL5. All of these were DSEs, which could form microsclerotia in *M. oiwakensis*. The growth response experiment revealed that inoculation of the plant with MoAL5 and CkDB5 promoted an increase in the total fresh weight of the seedlings. Chemical composition analysis showed that seedlings inoculated with CkDB5 had the highest berberine concentration. These results showed that some DSEs have the ability to promote growth and induce phytochemical responses in the host plant.

Keywords: berberine; *Cladophialophora chaetospira*; dark septate endophyte; growth response; *Hymenoscyphus*; *Mahonia oiwakensis*

1. Introduction

The *Mahonia* genus, a member of the Berberidaceae family, contains more than 60 species and is native to Asia and America [1]. Members of this genus include folk medicinal plants [2,3], and *Mahonia* species, in particular, possess antibacterial, antifungal and anti-inflammatory properties [4]. Many isoquinoline alkaloids, such as berberine, oxyanthine and tetrandrine, can be isolated from plants such as *M. aquifolium*. Of these, berberine possesses the best characteristics. Previous studies have shown that berberine can inhibit cell growth and induce apoptosis in several human cancer cell lines [5–10].

*M. oiwakensis* Hayata (*Alishan mahonia*) is an endemic species of Taiwan and a well-known folk medicinal plant. Wong et al. (2009) demonstrated that *M. oiwakensis* extract (MOE) inhibits the growth of human lung cancer cells in vitro and in vivo and suggested that it has therapeutic potential against human lung cancer [4].

Various forms of mycorrhizal fungi found in nature play important roles in plant nutrition and nutrient cycling [11]. Mycorrhizae promote host plant growth [12,13] and increase stress tolerance [14,15], thus accelerating the synthesis of secondary metabolites in host plants [16–22]. Many studies have reported the effects of mycorrhizae on the content of secondary metabolites in plants; however, most of these studies have focused on the effects of arbuscular mycorrhizal fungi (AMF) [16–18,20,21], though attention has been paid to dark septate endophytes (DSE) as well [19,22]. DSE can promote the uptake of nutrients (such as C, N, and P) of plants and also help plants against the survival stresses caused by biotic and abiotic factors [23]. Furthermore, only one of the AMF studies is related to berberine [16], and there are no reports about the effects of DSE on berberine
production in *M. oiwakensis*. Tan et al. (2016) demonstrated that two root-endophytic fungi, MoAL2 and MoAL5, can associate with *M. oiwakensis* seedlings [24]; however, the benefits for the host plant remain unknown. Among the four strains of root-endophytic fungi maintained in our laboratory, two (CkDB2 and CkDB5) have been identified as DSE and have been shown to promote the growth of the host plant [25]. It is well-known that DSEs can promote the growth and survival of the host plant; however, there is currently no research that is focused on the effects of DSEs on the berberine concentration. To the best of our knowledge, this is the first study to report the effects of DSEs on the growth promotion and berberine concentration in *M. oiwakensis*.

2. Results

2.1. Molecular Phylogenetic Analysis of the Four Strains

Taxonomic affinities, including the most closely matched sequences, were assigned to MoAL2, MoAL5, CkDB2 and CkDB5 based on BLAST sequence similarity analysis (Figure 1). Through ML (Maximum likelihood) analysis, the ITS (Internal transcribed spacer) sequence of MoAL5 was grouped with sequences of *Cladophialophora* and was found to be closely matched to *C. chaetospira*, with 89% bootstrap values and 100% ITS sequence similarity (Figure 1A). The ITS sequence of MoAL2 closely matched to *Hymenoscyphus repandus* and *H. menthae*, and MoAL2 was identified as a species of *Hymenoscyphus* (Figure 1A). CkDB2 was grouped with sequences of *Sporothrix* and was closely matched to *S. schenckii* (AF484468) (Figure 1B). CkDB5 was grouped with sequences of *Scolecobasidium humicola* and *Dactylaria purpurella* and was closely matched to species of *Ascomycota* (KX908468 and KX908411) with 95% bootstrap values (Figure 1B).

2.2. Morphology and Colonization in Resynthesized Seedlings

After three months of incubation, all treated seedlings survived (Figure 2A,C,E,G,I). The features of root associations for all treatments were observed by a light microscope (Figure 2B,D,F,H,J). *M. oiwakensi* seedlings inoculated with CkDB2 and CkDB5 grew well (Figure 2A,C), and dark septate microsclerotia-like structures were observed in the stained roots of CkDB2- and CkDB5-treated plants (Figure 2B,D). *M. oiwakensi* seedlings inoculated with MoAL2 and MoAL5 also grew well (Figure 2E,G), and hyaline microsclerotia-like structures were observed in the stained roots of MoAL2- and MoAL5-treated plants (Figure 2F,H). In the controls, the seedlings grew well (Figure 2I); however, no peculiarities were found in the stained roots (Figure 2J).
Figure 1. Cont.
2.3. Growth Responses

As shown in Table 1 and Figure 2A,C,E,G,I, the growth response analyses indicated that not every inoculation had a positive effect on plant growth after incubation for three months. The CkDB5-treated plants’ average shoot fresh weight (ASFW) (0.29 ± 0.13 g), average root fresh weight (ARFW) (0.24 ± 0.09 g), and average total fresh weight (ATFW) (0.53 ± 0.21 g) were significantly different from those of the control groups (p < 0.05) and were the highest among all the treated plants [ASFW: MoAL5-treated plants, 0.25 ± 0.07 g; MoAL2-treated plants, 0.15 ± 0.06 g; CkDB2-treated plants, 0.12 ± 0.06 g; and the control, 0.15 ± 0.06; ARFW: MoAL5-treated plants, 0.21 ± 0.07 g; MoAL2-treated plants, 0.10 ± 0.03 g; CkDB2-treated plants, 0.07 ± 0.03 g; and the control, 0.14 ± 0.05 g; ATFW: MoAL5-treated plants, 0.46 ± 0.13 g; MoAL2-treated plants, 0.25 ± 0.09 g; CkDB2-treated plants, 0.19 ± 0.08 g; and the control, 0.29 ± 0.10 g].

Table 1. Growth and berberine concentrations in M. oiwakensis seedlings inoculated with different fungal isolates after three months of incubation.

| Treatment | Shoot       | Root        | Total       | Berberine Concentration/µg g⁻¹ |
|-----------|-------------|-------------|-------------|-------------------------------|
| Control   | 0.15 ± 0.06 b<sup>c</sup> | 0.14 ± 0.05 b<sup>b</sup> | 0.29 ± 0.10 b<sup>c</sup> | 2419 ± 94 d<sup>d</sup> |
| CkDB2     | 0.12 ± 0.06 c<sup>c</sup> | 0.07 ± 0.03 b<sup>b</sup> | 0.19 ± 0.08 c<sup>c</sup> | 3140 ± 176 c<sup>c</sup> |
| CkDB5     | 0.29 ± 0.13 a<sup>a</sup> | 0.24 ± 0.09 a<sup>a</sup> | 0.53 ± 0.21 a<sup>a</sup> | 4441 ± 21 a<sup>a</sup> |
| MoAL2     | 0.15 ± 0.06 b<sup>c</sup> | 0.10 ± 0.03 b<sup>b</sup> | 0.25 ± 0.09 c<sup>c</sup> | 2890 ± 107 c<sup>c</sup> |
| MoAL5     | 0.25 ± 0.07 a<sup>b</sup> | 0.21 ± 0.07 a<sup>a</sup> | 0.46 ± 0.13 b<sup>b</sup> | 3809 ± 144 b<sup>b</sup> |

All values are means ± standard deviation of five replicates. Values in the same column with different letters (a, b, c, etc.) are different at 5% significance level.
Figure 2. Morphology of *M. oiwakensis* seedlings after incubation for 3 months: (A,C,E,G,I) show *M. oiwakensis* seedlings of all treatments (bar = 5 cm); (B,D,F,H,J) show the root stain for all treatments. (A,B): CkDB2-inoculation; (C,D): CkDB5-inoculation; (E,F): MoAL2-inoculation; (G,H): MoAL5-inoculation; (I,J): Control. Hyaline microsclerotia-like formations (arrows).

2.4. Berberine Concentration

The berberine concentrations determined from each treatment were shown to be significantly different from each other (Table 1 and Figure 3). All treated plants had higher berberine concentrations than the controls. The CkDB5-treated plants had the highest berberine concentration (4441 ± 21 µg/g) compared with the other treated plants (MoAL5-treated plants, 3809 ± 144 µg/g; CkDB2-treated plants 3140 ± 176 µg/g; MoAL2-treated plants, 2890 ± 107 µg/g; and the control, 2419 ± 94 µg/g).
3. Discussion

Although numerous sterile DSEs have been isolated from different plant roots, they have not been identified at the species level due to their inability to form teleomorphs and conidia [26,27]. However, in recent years, the ITS of rDNA has been successfully used to clarify the phylogenetic relationships and demonstrate the genetic diversity among DSEs [28–31]. According to the ITS analysis, CkDB2 was a newly recorded species (S. schenckii) from Taiwan. *S. schenckii* is distributed throughout the world and causes...
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with respect to root growth [25]. Additionally, based on our results, CkDB5 had similar
features with that in the controls. Between these two strains, CkDB5 was able to induce
AMF have been demonstrated to affect the types and concentrations of secondary metabo-
lites [58–60], and every species of AMF has a different effect on plants. For example,
DSEs were able to promote both of growth performance and berberine production in
M. oiwakensis [25] and facilitated increased production of berberine. One of the features of DSE is hyaline microsclerotia [46,47]. In this
study, staining of the roots revealed microsclerotia in the roots of all treated plants. Hence,
based on the above results, there is sufficient evidence to demonstrate that CkDB2, CkDB5,
MoAL2 and MoAL5 can be classified as DSEs and could associate with M. oiwakensis.

Mycorrhizal fungi, DSEs and plant growth-promoting rhizobacteria (PGPR) can pro-
more the growth response of their host plants [42,48–50]. DSEs are ascomycetes that can
facilitate the growth of their host plants without causing pathologies [42]. CkDB2 and
CkDB5 have been previously demonstrated to be DSEs and can promote the growth re-
spoonse in C. kanehirae [25]. In this study, CkDB2 and CkDB5 could act as a DSE with
M. oiwakensis and promote increased production of berberine in M. oiwakensis compared
with that in the controls. Between these two strains, CkDB5 was able to induce M. oiwakensis
in obtaining the highest value of fresh weight and could also induce the production of
the highest amount of berberine (1.83-fold higher than that of controls). Of the other two
strains (MoAL2 and MoAL5), MoAL5 was found to belong to C. chaetospira. However,
MoAL5 is different from the more common DSEs [51–53], which have no significant effects
on the fresh weight on plants such as the blueberry plant (Vaccinium corymbosum L.) [54].
As reported previously, several arbucular mycorrhiza fungi were also able to promote
berberine contents in Phellodendron chinense. For instance, the berberine contents in the root
of P. chinense seedlings were elevated 1.89-fold compared to control group after inoculated
with Glomus etunicatum for 3 months [16]. For the first time, our results demonstrated
that DSEs were able to promote both of growth performance and berberine production in
M. oiwakensis.

Colonization by fungi can cause a series of resistance reactions in host plants, includ-
ing eliciting an effect on secondary metabolites such as alkaloids and terpenoids. [55–57].
AMF have been demonstrated to affect the types and concentrations of secondary metabo-
lites [58–60], and every species of AMF has a different effect on plants. For example,
G. diaphanum, G. etunicatum, G. intraradices, Acaulospora mellea and A. laevis promote the
increased production of camptothecin in Camptotheca acuminata [33,59,60], whereas G. mani-
hot reduces it [35]. Furthermore, Zhou & Fan [16] have also shown that AMF can not only
promote growth but also increase the berberine content in P. chinense. Some DSEs are also
able to promote the increased production of flavonoids in S. involucrata seedlings [19]. In
this study, all four strains could facilitate increased production of berberine in M. oiwakensis.
On the other hand, some endophytic fungi (Alternaria sp. and Fusarium solani) have been
reported with the ability to produce berberine [61,62]. However, how DSE regulate the
berberine production in M. oiwakensis and its mechanism of action still need to investigate
in the future study. Among these four strains, CkDB5 (isolated from C. kanehirae) has
been demonstrated to have significant effects on the growth of C. kanehirae, particularly
with respect to root growth [25]. Additionally, based on our results, CkDB5 had similar
effects on M. oiwakensis. Therefore, it is safe to assume that CkDB5 has wide host-range compatibility and functional diversity.

4. Materials and Methods

4.1. Seeds

The seeds of M. oiwakensis were collected from the Alishan Recreational Park, Alishan Township, Chiayi County, Taiwan (120°48′45″ E, 23°30′46″ N, 2279 m altitude).

4.2. Strains

Four strains of DSEs were used in this study. Among these strains, MoAL2 and MoAL5 were isolated from M. oiwakensis [24], whereas CkDB2 and CkDB5 were isolated from Cinnamomum kanehirae [33]. These four strains were deposited at the Tree Mycorrhiza Laboratory of National Chiayi University. The internal transcribed spacer (ITS) genomic sequences of these four endophytes have been uploaded to GenBank (strains MoAL2 (Figure 4A), MoAL5 (Figure 4B), CkDB2 (Figure 4C) and CkDB5 (Figure 4D): KX509994, KX509995, KT780305 and KT780306, respectively).

![Figure 4. Colony morphology of these four strains on PDA medium. (A): MoAL2; (B): MoAL5; (C): CkDB2; (D): CkDB5.](image-url)
ected to NCBI MEGABLAST queries. Phylogenetic analysis was performed with MEGA 7.0 for maximum likelihood (ML) analysis based on the ITS sequences [67].

4.4. Inoculation with Endophytes

Inoculation was performed using the method of Ann, Tsai, Wang, & Hsien [68] and Zhang, Tang, Chen, & Wang [69] with some modifications. After cleaning, the seeds of *M. owakensis* were sterilized with 35% H$_2$O$_2$ for 3 min and rinsed three times with sterilized distilled water. The seeds were then transferred to a test pot containing a mixture of peat and vermiculite (1:1 v/v; previously sterilized at 121 °C for 60 min) for germination. The germinated seedlings were then transplanted to new tubes (4 cm in diameter, 18 cm in height) containing a mixture of peat and vermiculite (3:1 v/v; previously sterilized at 121 °C for 60 min) and were inoculated with the inoculum. These four strains grow on the PDA medium after 21-day incubation, and take out the edge of the colony for inoculum. For inoculation, each seedling was inoculated with two 5-mm diameter pieces of mycelium. Five treatments (one control and four inoculations) were used. Each treatment had five replicates. Each replicate was grown, watered and fertilized in the growth chamber (23 °C, 65% RH and 16:8-h light/dark cycle with 5000 lx as maximum illumination). This method is a kind of mycorrhizal synthesis that can avoid being affected by environmental microorganisms. After three-month incubations, the features of root associations for all treatments were observed using the method of staining root [15].

4.5. Plant Growth Responses

To measure the effects of these DSEs on the growth of the seedlings, the seedlings were carefully removed from their substrates after the incubation period, and their fresh weights were measured.

4.6. Determination of Berberine Concentrations

All seedlings were sliced into small pieces, air dried in a desiccator with silica gel to constant weight and then pulverized. For each sample, 2.5 mL of methanol was added to an aliquot of 250 µg powder. After 24 h, the mixture was filtered. The residue was rinsed twice with 1.0 mL methanol and pooled with the filter. The pooled solution then was subjected to a 3 mL-methanol, 3 mL-deionized-water pre-conditioned C18 solid-phase extraction cartridge (SPE, Strata C18-E, 55 µm, 70 A) and then flushed with 3 mL of 70% methanol. The eluents were collected and diluted with methanol to exactly 5.0 mL. An aliquot of 20 µL was injected into a high-performance liquid chromatography (HPLC) system for berberine determination. The conditions of HPLC used in the present study were described as below. In brief, the sample was separated by a reversed phase column (4.6 × 250 mm, 5 µm, Discovery C18 HPLC Column, Supelco) with a mobile phase flow rate of 0.8/mL/min, and the eluents were monitored at 260 nm by a UV detector (Agilent HPLC 1100 series, HP). The mobile phase comprised solvent A (methanol: CH$_3$CN, 1:4) and solvent B (2.5 mM CH$_3$COONH$_4$) and was ramped linearly from 10% A/90% B (0 min) to 20% A/80% B (15 min), 30% A/70% B (20 min), 35% A/65% B (40 min) and finally to 100% A/0% B (50 min). A calibration curve (y = 90.124; x−108.16, where y denotes the area signal of 260 nm and x denotes the berberine concentration in µg/mL), was prepared by six point-standard berberine solutions ranging in concentrations from 25 to 250 µg/mL. A correlation coefficient $R^2 = 0.9984$ was used to quantify the berberine concentration in the samples.

4.7. Statistical Analysis

Statistical Package for the Social Science (SPSS 12.0) (Chicago, IL, USA) for Windows was used to perform all statistical analyses. Tukey’s multiple range test at a significance level of $p \leq 0.05$ was used to analyze the differences among treatments.
5. Conclusions

This study demonstrated that CkDB2, CkDB5, MoAL2 and MoAL5 can associate with the roots of *M. oiwakensis*. Molecular analysis revealed that these four strains should be classified into four groups: CkDB2 belongs to *S. schenckii*; CkDB5 is a member of the Ascomycota; MoAL2 is a member of the genus *Hymenoscyphus*; and MaAL5 belongs to the *C. chaetospira*. Among these four strains, CkDB5 and MoAL2 are newly identified species globally, whereas CkDB2 and MoAL5 are newly identified species in Taiwan. MoAL5 and CkDB5 can help promote growth and increase the production of berberine in *M. oiwakensis*. With CkDB5 inoculation, berberine concentration can be increased by nearly 80%, so CkDB5 can potentially be used to increase the berberine concentration and promote the growth of *M. oiwakensis*.

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**References**

1. Ying, T.S.; Boufford, D.E.; Brach, A.R. **Berberidaceae.** In *Flora of China*; Wu, Z.Y., Raven, P.H., Hong, D.Y., Eds.; Science Press: Beijing, China; Missouri Botanical Garden Press: St. Louis, MO, USA, 2011; Volume 19, pp. 714–800.
2. Rohrer, U.; Kunz, E.M.; Lenkeit, K.; Schaffner, W.; Meyer, J. Antimicrobial activity of Mahonia aquifolium and two of its alkaloids against oral bacteria. *Schweiz. Mon. Zahnmed.* **2007**, *117*, 1126–1131.
3. Tseng, S.H.; Chien, T.Y.; Tseng, C.F.; Lin, Y.H.; Wu, C.H.; Wang, C.C. Prevention of hepatic oxidative injury by Xiao-Chen-Chi-Tang in mice. *J. Ethnopharmacol.* **2007**, *111*, 232–239. [CrossRef] [PubMed]
4. Wong, B.S.; Hsiao, Y.C.; Lin, T.W.; Chen, K.S.; Chen, P.N.; Kuo, W.H.; Chu, S.C.; Hsieh, Y.S. The in vitro and in vivo apoptotic effects of Mahonia oiwakensis on human lung cancer cells. *Chem. Biol. Interact.* **2009**, *180*, 165–174. [CrossRef]
5. Lin, J.P.; Yang, J.S.; Lee, J.H.; Hsieh, W.T.; Chung, J.G. Berberine induces cell cycle arrest and apoptosis in human gastric carcinoma SNU-5 cell line. *World J. Gastroenterol.* **2006**, *12*, 21–28. [CrossRef] [PubMed]
6. Kuo, H.P.; Chuang, T.C.; Tsai, S.C.; Tseng, H.H.; Hsu, S.C.; Chen, Y.C.; Kuo, C.L.; Kuo, Y.H.; Liu, J.Y.; Kao, M.C. Berberine, an isoquinoline alkaloid, inhibits the metastatic potential of breast cancer cells via Akt pathway modulation. *J. Agric. Food Chem.* **2006**, *54*, 3371–3378. [PubMed]
7. Liu, C.C.; Yang, J.S.; Chen, J.T.; Fan, S.; Yu, F.S.; Yang, J.L.; Lu, C.C.; Kao, M.C.; Huang, A.C.; Lu, H.F.; et al. Berberine induces apoptosis in human HSC-3 oral cancer cells via simultaneous activation of the death receptor-mediated and mitochondrial pathway. *Anticancer Res.* **2007**, *27*, 3371–3378. [PubMed]
8. Liu, C.H.; Tang, W.C.; Sia, P.; Huang, C.C.; Yang, P.M.; Wu, M.H.; Lai, I.L.; Lee, K.H. Berberine inhibits the metastatic ability of prostate cancer cells by suppressing epithelial-to-mesenchymal transition (EMT)-associated genes with predictive and prognostic relevance. *Int. J. Med. Sci.* **2015**, *12*, 63–71. [CrossRef]
9. Mantena, S.K.; Sharma, S.D.; Katiyar, S.K. Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. *Mol. Cancer Ther.* **2006**, *5*, 296–308. [CrossRef]
10. Mantena, S.K.; Sharma, S.D.; Katiyar, S.K. Berberine inhibits growth, induces G1 arrest and apoptosis in human epidermoid carcinoma A431 cells by regulating Cdki-Cdk-cyclin cascade, disruption of mitochondrial membrane potential and cleavage of caspase 3 and PARP. *Carcinogenesis* **2006**, *27*, 2018–2027. [CrossRef]
11. Cairney, J.W.G.; Meharg, A.A. *Eriocymycorrhiza*: A partnership that exploits harsh edaphic conditions. *Eur. J. Soil Sci.* **2003**, *54*, 735–740. [CrossRef]
12. Schmid, E.; Oberwinkler, F.; Gomez, L.D. Light and electron microscopy of a host-fungus interaction in the roots of some epiphytic ferns from Costa Rica. *Can. J. Bot.* **1995**, *73*, 991–996. [CrossRef]
13. Azcón-Aguilar, C.; Barea, J.M. Applying mycorrhiza biotechnology to horticulture: Significance and potentials. *Sci. Hortic.* **1995**, *58*, 1–24. [CrossRef]
14. Gibson, B.R.; Mitchell, D.T. Influence of pH on copper and zinc sensitivity of ericoid mycobionts in vitro. *Mycorrhiza* **2005**, *15*, 231–234. [CrossRef]
15. Lin, L.C.; Lee, M.J.; Chen, J.L. Decomposition of organic matter by the ericoid mycorrhizal endophytes of Formosan rhododendron. *Mycorrhiza* **2011**, *21*, 331–339. [CrossRef] [PubMed]
16. Zhou, J.H.; Fan, J.H. Effects of AM fungi on the berberine content in Phellodendron chinense seedings. *North. Hortic.* 2007, 12, 25–27.

17. Venkateswarlu, B.; Pirat, M.; Kishore, N.; Rasul, A. Mycorrhizal inoculation in neem (*Azadirachta indica*) enhances azadirachtin content in seed kernels. *World J. Microbiol. Biotechnol.* 2008, 24, 1243–1247. [CrossRef]

18. Karthikeyan, A.; Shanthi, V.; Nagasathaya, A. Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica* L. *Int. J. Green Pharm.* 2009, 3, 78–80. [CrossRef]

19. Wu, L.; Lv, Y.; Meng, Z.; Chen, J.; Guo, S.X. The promoting role of an isolate of dark-septate fungus on its host plant *Saussurea involucrata* Kar. et Kir. *Mycorrhiza* 2010, 20, 127–135. [CrossRef]

20. Zeng, Y.; Guo, L.P.; Chen, B.D.; Hao, Z.P.; Wang, J.Y.; Huang, L.Q.; Yang, G.; Cui, X.M.; Yang, L.; Wu, Z.X.; et al. Arbuscular mycorrhizal symbiosis and active ingredients of medicinal plants: Current research status and perspectives. *Mycorrhiza* 2013, 23, 253–265. [CrossRef]

21. Zubek, S.; Rola, K.; Szweczyk, A.; Majewksa, M.L.; Turnau, K. Enhanced concentrations of elements and secondary metabolites in *Mahonia oiwakensis* and its dark septate endophytes. *Plant Soil* 2015, 390, 129–142. [CrossRef]

22. He, C.; Cui, J.; Chen, X.; Wang, W.; Hou, J. Effects of enhancement of liquorice plants with dark septate endophytes on the root growth, glycyrrhizic acid and glycyrrhizin accumulation amended with organic residues. *Curr. Plant Biol.* 2020, 23, 100154. [CrossRef]

23. Hou, L.; Yu, J.; Zhao, L.; He, X. Dark Septate Endophytes Improve the Growth and the Tolerance of Medicago sativa and Ammopiptanthus mongolicus under Cadmium Stress. *Front. Microbiol.* 2020, 10, 3061. [CrossRef] [PubMed]

24. Tan, Y.L.; Chang, T.P.; Chen, J.L.; Ku, K.L.; Lin, L.C. Morphology of root-fungus association of *Mahonia oiwakensis* and its endophytes. *Q. J. Chin. For.* 2016, 49, 1–12. (In Chinese)

25. Lin, L.C. Growth effect of *Cinnamomum kanehirae* cuttings associated with its dark septate endophytes. *Pak. J. Biol. Sci.* 2016, 19, 299–305. [CrossRef] [PubMed]

26. Newsham, K.K. *Phialophora graminicola*, a dark septate fungus, is a beneficial associate of the grass *Vulpia ciliata* ssp. *ambigua*. *New Phytol.* 1999, 144, 517–524. [CrossRef]

27. Piercey, M.M.; Graham, S.W.; Currah, R.S. Patterns of genetic variation in *Phialocephala fortinii* across a broad latitudinal transect in Canada. *Mycol. Res.* 2004, 108, 955–964. [CrossRef] [PubMed]

28. Liu, G.; Chambers, S.M.; Cairney, J.W.G. Molecular diversity of ericoid mycorrhizal endophytes isolated from *Woollsia pungens*. *New Phytol.* 1998, 140, 145–153. [CrossRef]

29. Chambers, S.M.; Williams, P.G.; Seppelt, R.D.; Cairney, J.W.G. Molecular identification of *Hymenoscyphus* sp. from rhizoids of the leafy liverwort *Cephalozia exiliflora* in Australia and Antarctica. *Mycol. Res.* 1999, 103, 286–288. [CrossRef]

30. McLean, C.B.; Cunnington, J.H.; Lawrie, A.C. Molecular diversity within and between ericoid endophytes from the Ericaceae and Epacridaceae. *New Phytol.* 1999, 144, 351–358. [CrossRef]

31. Sharples, J.M.; Chambers, S.M.; Meharg, A.A.; Cairney, J.W.G. Genetic diversity of root-associated fungal endophytes from *Calluna vulgaris* at contrasting field sites. *New Phytol.* 2000, 148, 157–162. [CrossRef] [PubMed]

32. Barros, M.B.; de Almeida Paes, R.; Schubach, A.O. *Sporothrix schenckii* and *Sporothrix schenckii* var. *Sporothrix schenckii* var. *Sporothrix schenckii* var. [CrossRef] [PubMed]

33. Lin, L.C.; Chang, T.P.; Hong, S.L.; Hsieh, C.K.; Chen, J.L.; Tseng, T.Y. The compatibility of *Cinnamomum kanehirae* cuttings and its dark septate endophytes. *Q. J. Chin. For.* 2015, 48, 127–136. (In Chinese)

34. De Meyer, E.M.; de Beer, Z.W.; Summerbell, R.C.; Moharram, A.M.; de Hoog, G.S.; Vismer, H.F.; Wingfield, M.J. Taxonomy and phylogeny of new wood- and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia* 2008, 100, 647–661. [CrossRef]

35. Zhao, X.; Yu, T.; Wang, Y.; Yan, X.-f. Effect of arbuscular mycorrhiza on the growth of *Camptotheca acuminata* seedlings. *J. For. Res.* 2006, 17, 121–123. [CrossRef]

36. Meriden, Z.; Marr, K.A.; Lederman, H.M.; Ille, P.B.; Villa, K.; Riedel, S.; Carroll, K.C.; Zhang, S.X. Ochroconis gallopava infection in a patient with chronic granulomatous disease: Case report and review of the literature. *Med. Mycol.* 2012, 50, 883–889. [CrossRef]

37. Fukushima, R.; Udagawa, S.; Kawashima, Y.; Kawamura, Y. Subcutaneous abscesses caused by *Ochroconis gallopavum*. *J. Med Vet. Mycol.* 1996, 24, 175–182. [CrossRef]

38. Sides, E.H.; 3rd; Benson, J.D.; Padhye, A.A. Phaeohyphomycotic brain abscess due to *Ochroconis gallopavum* in a patient with malignant lymphoma of a large cell type. *J. Med Vet. Mycol.* 1991, 29, 317–322. [CrossRef]

39. Shoham, S.; Pic-Aluas, L.; Taylor, J.; Cortez, K.; Rinaldi, M.G.; Shea, Y.; Walsh, T.J. Transplant-associated *Ochroconis gallopavum* infections. *Transpl. Infect. Dis.* 2008, 10, 442–448. [CrossRef]

40. Mayer, N.; Bastani, B. A case of pulmonary cavitary lesion due to *Dactylaria constricta* var. *gallopava* in a renal transplant patient. *Nephrol. Carlton* 2009, 14, 262. [CrossRef]

41. Crous, P.W.; Schubert, K.; Braun, U.; de Hoog, G.S.; Hocking, A.D.; Shin, H.D.; Groenewald, J.Z. Opportunistic, human-pathogenic species in the Herpotrichiellaceae are phenotypically similar to saprobes or phytopathogenic species in the Venturiaceae. *Stud. Mycol.* 2007, 58, 185–217. [CrossRef]

42. Jumpponen, A.; Trappe, J.M. Dark septate endophytes: A review of facultative biotrophic root-colonizing fungi. *New Phytol.* 1998, 140, 295–310. [CrossRef]
43. Ohki, T.; Yonezawa, M.; Hashiba, T.; Masuya, H.; Usuki, F.; Narisawa, K.; Narisawa, K. Colonization process of the root endophytic fungus *Heterocentrum chaetospira* in roots of Chinese cabbage. *Mycoscience* 2002, 43, 191–194. [CrossRef]

44. Narisawa, K.; Tokumasu, S.; Hashiba, T. Suppression of clubroot formation in Chinese cabbage by the root endophytic fungus, *Heterocentrum chaetospira*. *Plant Pathol.* 1998, 47, 206–210. [CrossRef]

45. Narisawa, K.; Ohki, K.T.; Hashiba, T. Suppression of clubroot and *Verticillium* yellows in Chinese cabbage in the field by the root endophytic fungus, *Heterocentrum chaetospira*. *Plant Pathol.* 2000, 49, 141–146. [CrossRef]

46. Zhang, Y.; Li, T.; Li, L.; Zhao, Z. The colonization of plants by dark septate endophytes (DSE) in the valley-type savanna of Yunnan, southwest China. *Afr. J. Microbiol. Res.* 2011, 5, 5540–5547. [CrossRef]

47. Lukesova, T.; Kohout, P.; Vetrovsky, T.; Vohnik, M. The potential of Dark Septate Endophytes to form root symbioses with ectomycorrhizal and ericoid mycorrhizal middle European forest plants. *PLoS ONE* 2015, 10, e0124752. [CrossRef]

48. Shivanna, M.B.; Meera, M.S.; Hyakumachi, M. Sterile fungi from zoysiagrass rhizosphere as plant growth promoters in spring wheat. *Can. J. Microbiol.* 1994, 40, 637–644. [CrossRef]

49. Fernando, A.A.; Currah, R.S. A comparative study of the effects of the root endophytes *Leptodontidium orchidicola* and *Phialocephala fortinii* (Fungi Imperfecti) on the growth of some subalpine plants in culture. *Can. J. Bot.* 1996, 74, 1071–1078. [CrossRef]

50. Bent, E. Induced systemic resistance mediated by plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF). In *Multigenic (Fungi Imperfecti) on the growth of some subalpine plants in culture.* Springer: Boston, MA, USA, 2006; pp. 225–258.

51. Harman, G.E.; Howell, C.R.; Viterbo, A.; Chet, I.; Lorito, M. *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* 2004, 2, 43–56. [CrossRef]

52. Narisawa, K.; Hambleton, S.; Currah, R.S. *Heterocentrum chaetospira*, a dark septate root endophyte allied to the Herpotrichiellaceae (Chaetothyriales) obtained from some forest soil samples in Canada using bait plants. *Mycoscience* 2007, 48, 274–281. [CrossRef]

53. Vano, I.; Sakamoto, K.; Inubushi, K. Phylogenetic relationships among non-pathogenic isolates of dark septate endophytes from Ericaceae plants. *HortResearch* 2011, 65, 41–47.

54. Vano, I.; Sakamoto, K.; Inubushi, K. Selection of dark septate endophytes from Ericaceae plants to enhance blueberry (*Vaccinium corymbosum L.*) seedling growth. In Proceedings of the 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia, 1–6 August 2010.

55. Petrini, O. (Ed.) *Fungal Endophytes of Tree Leaves*; Springinger: New York, NY, USA, 1991; pp. 179–197.

56. Giovannetti, M.; Sbrana, C. Meeting a non-host: The behaviour of AM fungi. *Mycorrhiza* 1998, 8, 123–130. [CrossRef]

57. Brundrett, M.C. Coevolution of roots and mycorrhizas of land plants. *New Phytol.* 2002, 154, 275–304. [CrossRef]

58. Pongrac, P.; Vogel-Mikus, K.; Regvar, M.; Tolra, R.; Poschenrieder, C.; Barcelo, J. Glucosinolate profiles change during the life cycle and mycorrhizal colonization in a Cd/Zn hyperaccumulator *Thlaspi praecox* (*Brassicaceae*) seedling growth. In Proceedings of the 19th World Congress of Soil Science, Soil Solutions for a Changing World, Beijing, China, 2008, 10, 22

59. Yadav, K.; Aggarwal, A.; Singh, N. Arbuscular mycorrhizal fungi (AMF) induced acclimatization, growth enhancement and colchicine content of micropropagated *Gloriosa superba* L. plantlets. *Ind. Crop. Prod.* 2015, 45, 88–93. [CrossRef]

60. Duan, L. Isolation and Identification of Producing Endophytic Fungi of Berberine from the Plant *Phellodendron amurense*. *Plant Soil* 2010, 326, 3–20. [CrossRef]

61. Smith, S.E.; Facelli, E.; Pope, S.; Andrew Smith, F. Plant performance in stressful environments: Interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant Soil* 2010, 326, 3–20. [CrossRef]

62. Spiral, O. (Ed.) *Fungal Endophytes of Tree Leaves*; Springer: New York, NY, USA, 1991; pp. 179–197.

63. Miller, E.M.; Bahnweg, G.; Sandermann, H.; Geiger, H.H. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Res.* 1992, 20, 6115–6116. [CrossRef] [PubMed]

64. Sigler, L.; Allan, T.; Lim, S.R.; Berch, S.; Berbee, M. Two new *Cryptosporiopsis* species from roots of ericaceous hosts in western North America. *Stud. Mycol.* 2005, 53, 53–62. [CrossRef]

65. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* 1994, 172, 4238–4246. [CrossRef] [PubMed]

66. De Hoog, G.S.; Gerrits van den Ende, A.H. Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* 1998, 41, 183–189. [CrossRef] [PubMed]

67. Thompson, J.D.; Gibson, T.J.; Plewniak, F.; Jeanmougin, F.; Higgins, D.G. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 1997, 25, 4876–4882. [CrossRef] [PubMed]

68. Ann, P.; Tsai, J.N.; Wang, I.T.; Hsien, M.L. Response of fruit trees and ornamental plants to brown root rot disease by artificial inoculation with *Phellinus noxius*. *Plant Pathol.* 1999, 8, 61–66.

69. Zhang, H.H.; Tang, M.; Chen, H.; Wang, Y.J. Effects of a dark-septate endophytic isolate LBF-2 on the medicinal plant *Lycium barbarum* L. *J. Microbiol.* 2012, 50, 91–96. [CrossRef] [PubMed]