The mycorrhizal fungi of *Cymbidium* promote the growth of *Dendrobium officinale* by increasing environmental stress tolerance

Yulong Li [1], **Zhihua Kang** [2], **Xia Zhang** [3], **Ping Sun** [4], **Xiaohui Jiang** [5], **Zhengmin Han** [Corresp. 1]

[1] College of Forestry, Nanjing Forestry University, Nanjing, China

[2] Jiangsu Academy of Agricultural Sciences, Nanjing, China

[3] Suqian Forest Pest Quarantine Control Station, Suqian, China

[4] Jiangsu Aosaikang Pharmaceutical Co., Ltd, Suzhou, China

[5] Garden Bureau, Management Committee of Huangshan Scenic Area, Huangshan, China

Corresponding Author: Zhengmin Han
Email address: zmhan_njfu@163.com

Dendrobium officinale is a medicinal herbal plant with important health care value and high demand. Due to its slow growth and scarcity in nature, its yield depends on intensified cultivation. During cultivation, biotic and abiotic stresses were important factors of production loss. In this study, the effects on *D. officinale* of four mycorrhizal fungi isolated from *Cymbidium* were tested. The obviously higher mass yield of the treated plants in medium and pots indicated the growth promotion effect of the fungi. Furthermore, an abiotic stress test indicated stronger drought tolerance among the treated plants. For the biotic stress test, two root rot pathogens, *Fusarium solani* and *Fusarium graminearum* , were isolated and identified from root rot of *D. officinale*. In an *in vitro* inhibition test, the four mycorrhizal fungi could resist the growth of these pathogens. In vivo studies showed that these four mycorrhizal fungi could improve the survival rate and fresh weight and decrease the root rot rate of pathogen-inoculated seedlings. The four *Cymbidium* mycorrhizal fungi are compatible strains for improving the growth rate of *D. officinale* by increasing its environmental stress tolerance, providing an effective way to supply resources through artificial reproduction.

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2   # growth of *Dendrobium officinale* by increasing

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4   **Yulong Li[1], Zhihua Kang[2], Xia Zhang[3], Ping Sun[4], Xiaohui Jiang[5], Zhengmin Han[1*]**
5   [1]Department, Faculty, University name, Town, State
6   [2]Department, Institution name, Town, State
7   [1]College of Forestry, Nanjing Forestry University, Nanjing, China.
8   [2]Jiangsu Academy of Agricultural Sciences, Nanjing, China
9   [3]Suqian Forest Pest Quarantine Control Station, Suqian, China.
10 [4]Jiangsu Aosaikang Pharmaceutical Co., Ltd, Nanjing, China.
11 [5]Garden Bureau, Management Committee of Huangshan Scenic Area, Huangshan, China
12 Corresponding author
13 Zhengmin Han
14 College of Forestry, Nanjing Forestry University, NO.59, Longpan road Nanjing, 210036, China.
15 Email address: zmhan_njfu@163.com

Abstract

18   *Dendrobium officinale* is a medicinal herbal plant with important health care value and high
19 demand. Due to its slow growth and scarcity in nature, its yield depends on intensified
20 cultivation while biotic and abiotic stresses were important factors that causes production loss.
21 Orchidaceae can form association with rhizoctonias collectively, and studies have found that
22 some orchids showed a high level of strain-species specificity to orchid mycorrhizal fungi
23 (OMF), yet the specificity of OMF on *D. officinale* needs to explored. In this study, the effects on
24 *D. officinale* of four OMF isolated from *Cymbidium* were tested. The obviously higher mass yield
25 of the treated plants in medium and pots indicated the growth promotion effect of the fungi.
26 Furthermore, an abiotic stress test indicated stronger drought tolerance among the treated
27 plants. For the biotic stress test, two root rot pathogens, *Fusarium solani* and *Fusarium*
28 *graminearum*, were isolated and identified from root rot of *D. officinale*. In an *in vitro* inhibition
29 test, the four OMF could resist the growth of these pathogens. In vivo studies showed that these
30 four OMF could improve the survival rate and fresh weight and decrease the root rot rate of
31 pathogen-inoculated seedlings. The four OMF namely; *Hyphomycete* sp., *Umbelopsis* sp.,
32 *Ceratorhiza* sp. and *Ceratorhiza* sp. are compatible strains for improving the growth rate of *D.*
33 *officinale* by increasing its environmental stress tolerance, providing an effective way to supply
34 resources through artificial reproduction.

Introduction

36   *Dendrobium officinale* Kimura et Migo belongs to *Orchidaceae* and has high medicinal
37 value. It has been widely used in Asian countries for hundreds of years to enhance immunity,
38 provide antithrombotic and antineoplastic effects, and delay ageing[1-4]. Due to the rising
39 demand for this herb, wild *D. officinale* is severely exploited and will become extinct. Currently,

40 massive amounts of *D. officinale* are rapidly propagated under intensified cultivation to
41 overcome this problem[5]. However, issues in artificial cultivation have also emerged, such as
42 low growth rate, poor adaptability and plant disease. Notably, susceptibility to withering and root
43 and stem rot mainly caused by fungal disease has resulted in serious economic losses in the
44 cultivation of this plant [6, 7].
45   Mycorrhizal fungi are a group of fungi that live in healthy plants at a certain or all life stages
46 [8, 9]. Mycorrhizal fungi can form a mutually beneficial relationship with host plants. Orchid
47 mycorrhizal fungi (OMF) are present in all orchid species and play an important role in the
48 growth of orchid species by establishing obligate relationships conducive to gain carbon,
49 nutrients and water, especially during early life stages when orchids lack sufficient nutrient
50 reserves in seeds [10, 11]. While orchid acquires nutrients by mycoheterotrophy, the root
51 function of the host can be strengthened by the OMF to increase the absorbability of mineral
52 elements, especially phosphorus [12]. OMF can also stimulate the growth and development of
53 host plants, improving the host's resistance to stress [13, 14]. Applying suitable OMF could be
54 advantageous for orchid conservation and reproduction, e.g., propagation, ex situ seeding or
55 germination. However, the acceptability of and preference for OMF by the host is an important
56 factor to consider before application [15-17]. Mycorrhizal fungi may have different effects on
57 different hosts, which may depend on the process of recognition between the host and
58 mycorrhizal fungus as well as compliance with symbiosis [18-20]. Suitable mycorrhizal fungi are
59 needed for seed germination and seedling growth of *D. officinale* under natural conditions [21,
60 22]. Even though adequate nutrition is provided by artificial cultivation, this plant is slow-growing
61 and sensitive to biological and abiotic stressors. This is probably because appropriate
62 mycorrhizal fungi are deficient in artificial cultivation conditions.
63   Root rot is a major disease of *D. officinale* that is mainly caused by fungal pathogens. It
64 can weaken the root function of plants until the whole plant dies [23]. This disease can cause
65 massive infection and death in intensive cultivation of *D. officinale* [6, 7]. Fungal pathogens in
66 *Dendrobium* have been identified, such as *Fusarium* sp., *Pythium* sp. and *Ceratobasidium* sp.
67 [6, 7, 24]. Chemical agents *s*uch as metalaxyl-M, propamocarb, and Bordeaux mixtures have
68 been widely used to prevent and treat this disease [25]. However, chemical residues on the
69 products inhibit the growth of probiotic fungi and lead to low-quality products that are harmful to
70 sustainable agricultural development [26, 27].
71   In view of the above problems in intensified cultivation, four OMF namely; GDB162,
72 GDB254, GS222, MLX102 isolated from other *Cymbidium* were used to promote the growth and
73 to increased abiotic and biotic stress tolerance of *D. officinale* because these strains reportedly
74 showed growth promotion effect on *Cymbidum* such as *C. hybrid*, *C. goeringii, C. mastersii* in
75 former studies [28-30]. On the other side, the pathogenic fungi isolated and identified due to the
76 root disease is still a problem in intensified cultivation of *D. officinale* [6, 7]. Further, these OMF
77 were used to prevent and control root rot *D. officinale*. Our findings have great significance in
78 increasing and reducing the morbidity of *D. officinale* under intensive cultivation.
79 **Materials & Methods**

Plant materials, fungi and medium

The four mycorrhizal fungi are GDB162 (*Hyphomycete* sp.) [30], GDB254 (*Umbelopsis* sp.) [30], GS222 (*Ceratorhiza* sp.) [30, 31] originating from *Cymbidium faberi* Rolfe in Anhui Province, China, and MLX102 (*Ceratorhiza* sp.) [28-30], which was isolated from *Cymbidium sinense* (Jackson ex Andrews) Willd in Yunnan Province, China. *D. officinale* with root disease was collected from a nursery in Wujiang city, Jiangsu Province, China. Fungi were incubated on PSA medium containing 20 g sucrose, 20 g agar, 200 g potato and 1000 mL water. Mycorrhizal fungal infection of *D. officinale* was facilitated by DE symbiotic medium [32] containing K_2SO_4 1.0 mg/L, $FeSO_4$ 100 mg/L, $MnCl_2$ 3.3 mg/L, $NaMoO_4$ 1.0 mg/L, $MgSO_4$ 0.5 mg/L, KH_2PO_4 0.4 mg/L, H_3BO_4 25 mg/L, $ZnSO_4$ 2.8 mg/L, yeast extract 10 g/L, 9 g/L soluble starch and 4.5 g/L agar powder (pH 6.0). Murashige and Skoog (1962) (MS) medium was used as a medium for the growth of *D. officinale* seedlings[33]. The rooting medium was MS medium plus 1 mg/L NAA (1-naphthylacetic acid).

The plant effects of mycorrhizal fungi on *D. officinale*

D. officinale PLBs were cultured in rooting medium. After that, three seedlings with similar growth conditions (approximately 1.5 g of each) were transferred to solid DE symbiotic medium and cultured at 25°C for two days to verify the absence of contamination. Three 5 mm diameter blocks of agar containing fungi were evenly inoculated around *D. officinale* seedlings. For the testing of tissue culture seedlings, the seedlings were cultured at 25°C and 12 h daily light for 60 days. Then, fresh weight, chlorophyll content, stem polysaccharide levels, and root acid phosphatase activity (ACP) were measured and analyzed. For the testing of pot seedlings, the seedlings were transplanted to the pots after 15 days of OMF inoculation and were grown in a closed green house with natural light at 25°C for 5 months. Then, the survival rate, stem length, stem diameter and leaf number were measured and analyzed. The experiment was performed in thirty replicates.

The effect of mycorrhizal fungi on plant drought resistance

D. officinale seedlings in pot with similar growth condition and weight were selected for testing of drought resistant. The 4 leaves (about 5 mm around wide) of *D. officinale* infected by mycorrhizal fungi were placed around the root of the seedling for mycorrhizal fungi inoculation and the CK group consist of leaves without mycorrhizal fungi incubation [34, 35]. The mycorrhizal fungi infected the root of the plant and adequate water was supplied. Then, three experimental groups were set up. The group I (CK) was adequately water was supplied every week for the whole time. The group II was subjected to limited water supply every week, and the group III was subjected to adequate water was supplied only once at the beginning. After that, the survival rate of the plant of group III was recorded at 15, 30, 60 days and the MDA content of group I and II were measured and analyzed at 60 days. Each group had 10 replicate. Malonaldehyde (MDA) content was determined with the method of thibabituric acid (TBA) by using an MDA assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China, cat: A003-1-2) according to the manufacturer's instructions and determined at 532 nm. The experiments were performed in triplicate.

120 Determination of chlorophyll, and phosphatase activity

121   Chlorophyll a and b were determined as described previously [36]. Fragmented fresh leaves
122 (0.25 g) were transferred into a mortar, and then 2.5 ml of acetone was added. The mixture was
123 ground to a homogenate, and 2.5 ml of 80% acetone was added. The samples were centrifuged
124 at 12,000 x g for 10 min. The volume of the supernatant was adjusted to 10 ml with 80% acetone.
125 Then, 0.5 ml chlorophyll extract was diluted with 2 ml of 80% acetone, and the solution was
126 transferred to a cuvette. The 80% acetone solution was used as a control. The absorbance was
127 measured by a spectrophotometer at 645 and 663 nm to determine the chlorophyll a and b
128 contents, respectively.
129   Acid phosphatases activity was assayed using ρ-nitrophenol. Fresh root (0.5 g) was
130 transferred to a mortar and pestle at 0-4°C in 50 mM sodium acetate buffer (pH 5.5). The sample
131 were centrifuged at 12000 g for 15 min and the supernatant collected. ACP was determined using
132 ρ-nitrophenol phosphate as substrate and measuring the amount of ρ-nitrophenol produced.
133 Activity was quantified by comparing the absorption at 410 nm to a standard curve of diluted ρ-
134 nitrophenol solutions and NaOH. One unit of phosphatase is equivalent to the amount of enzyme
135 producing 1 μM of product per min under assay conditions.

136 Pathogen isolation and identification

137   The pathogens were isolated from rotted roots of *D. officinale*. The rotted roots were
138 soaked in 70% ethanol for 5-10 seconds, then in 0.1% mercury bichloride for 90 seconds and
139 washed with sterilized water three times. The treated roots were incubated on PSA medium at
140 26°C for 3-5 days. All suspected mycelium tips of pathogens were transferred to new PDA
141 medium. The following three primer pairs were used to amplify the fungal rRNA internal
142 transcribed spacer (ITS), the second largest RNA polymerase subunit (RPB2) and large
143 fragment of ribosomal (LSU) fragments: ITSF (5'- TCCGTAGGTGAACCTGCGG-3')/ ITSR (5'-
144 TCCTCCGCTTATTGATATGC-3') [37], RPB2-5F2(5' - GGGGWGAYCAGAAGAAGGC)/7cR (5'
145 - CCCATRGCTTGYTTRCCCAT) [38] and LSUF LSUF (5'- ATCCTGAGGGAAACTTC-3')/
146 LSUR (5' - GTACCCGCTGAACTTAAGC-3') [39].

147 Pathogenicity test

148   A pathogenicity test was performed on *D. officinale* by following Koch's postulates with
149 tissue culture seedlings and plants in pots. The seedlings were incubated on DE medium for 2
150 weeks at 26°C with a 12-h dark/light cycle. The pathogenic fungi were evenly inoculated around
151 seedlings for 30 days of co-incubation. The plants in pots containing dried bark and peat were
152 grown at a ratio of 2:5 at 26° C for 15 days. The fungi were incubated in Czapek medium with
153 120 rpm shaking and 25° C for 4 days. Then, the conidia suspensions (10^6 CFU/ml) were
154 created and inoculated into the rhizosphere of plants in pots. The plants were incubated at 26°C
155 and 65% relative humidity for 30 days, and the root rot rate and death rate were recorded. The
156 root rot severity was rated on a scale of 1 (root no wilting, white and plump) to 5 (plant death,
157 root complete yellow and shrinking) [40]. The experiments were performed in triplicate.

Antagonism studies *in vitro*

158

159   The mycorrhizal fungi and pathogens were inoculated on PSA medium (2 cm away from
160   the edge in a 9 cm petri dish) at an interval of 5 cm [41, 42]. The inoculated plate was incubated
161   at 20°C for 1 week, and the colony diameters of both fungi were measured every 24 hours. The
162   experiments were performed in triplicate.

The effect of mycorrhizal fungi on pathogens

163

164   Three 5 mm diameter blocks of agar containing OMF were evenly inoculated around *D.*
165   *officinale* seedlings respectively. Then, the seedlings were incubated at 25°C under 12 h daily
166   light for 15 days. After that, the co-incubated seedlings were transferred to pots in a
167   greenhouse, and the seedlings were inoculated with 1 mL of the conidia suspensions (10^6
168   CFU/ml) of the pathogenic fungi. The survival rate, total root rot and flesh weight were recorded
169   after 30 days. Ten replicates of the experiment were performed.

Statistical analysis

170

171   Statistical analyses were performed using SPSS 17.0. One-way analysis of variance
172   (ANOVA) and Tukey's test were used to detect the differences in seedling weight,
173   polysaccharide rate, chlorophyll content, acid phosphatase activity, MDA content, colony
174   diameter and root rot rate, survival rate.

175

176   **Results**

The promotion of growth of tissue-cultured *D. officinale* by mycorrhizal fungi

177

178   Four mycorrhizal fungi were inoculated to test their growth-promoting effect on tissue-
179   cultured *D. officinale.* The results showed that these four strains promoted the growth of *D.*
180   *officinale* for 60 days. The seedlings demonstrated better growth, with bright green shoots (Fig.
181   1), higher fresh seeding weight (Fig 2A), higher chlorophyll contents (Fig 2B), and higher levels
182   of polysaccharide in the stems (Fig 2C) compared with those in the control group (CK). The
183   differences between the stimulatory effects of GS222, GDB254, and MLX102 on the pigment
184   contents were not significant. MLX102 had the strongest promoting effect on chlorophyll a levels,
185   which demonstrated a 1.85-fold increase compared to that in the CK. GDB254 was the most
186   effective stimulator of chlorophyll b (2.72-fold) and total chlorophyll (1.94-fold) contents compared
187   to those in the CK. GDB254-treated plants had a greater increase in weight. The acid
188   phosphatase activity (Fig 2D) indicated that the phosphorus absorption of inoculated *D. officinale*
189   roots was weaker than that in the CK roots.

The promotion of growth of *D. officinale* in pots by mycorrhizal fungi

190

191   To study the promoting effect of these mycorrhizal fungi on *D. officinale*, four fungi were
192   inoculated into cultivated seedlings in pots. The results showed that all the mycorrhizal fungi had
193   a growth-promoting effect on the seedlings after for 5 months. The seedling survival rate after
194   transplantation from the medium to the substrate in the GDB254, MLX102, GS222, and GDB162

195   groups was increased by approximately 16%, 13%, 10%, and 13%, respectively, compared with
196   that in the CK group (Fig. 3A). The stem diameter, stem height, and leaf number of the seedlings
197   inoculated with mycorrhizal fungi (Fig. 3 B, C, D) were significantly increased. GDB162 had the
198   most efficient growth-promoting effect of all mycorrhizal fungi and induced an increase in seedling
199   thickness, height, and leaf number by approximately 118%, 54%, and 58%, respectively.

Drought stress resistance of *D. officinale* due to mycorrhizal fungi

201   To evaluate the effect of these fungi on the drought resistance of *D. officinale*, the survival
202   rate and MDA (malondialdehyde) content were measured under various drought conditions. The
203   results showed that the survival rate of *D. officinale* was significantly improved in the 15, 30, 60
204   days of drought conditions in the presence of mycorrhizal fungi (Fig. 4A). In the GDB162,
205   GS222, and GDB254 tested groups, the survival rate of *D. officinale* at various drought periods
206   was improved by more than 40% compared with the CK group. On the other hand, the MDA
207   content in mycorrhizal fungi inoculation groups was steadier and maintained at a low level in the
208   drought period,   while the MDA content of the CK group during drought is increased to twice
209   times that of the normal growth environment. This phenomenon was more obvious in the
210   MLX102 and GDB254 groups than in the other groups (Fig. 4B). In addition, most of seedlings
211   in group III were dead, hence, MDA content analysis was not conducted for this group. The
212   results indicated that the four mycorrhizal fungi could promote drought resistance in *D.
213   officinale*.

Isolation and identification of pathogenic fungi

215   Two fungi, TS1 and TS2, were isolated from *D. officinale* with root rot and showed
216   pathogenicity (Figure 5). The sequence data of TS1 showed 100% homology to *Fusarium solani*
217   (GenBank accessions ITS-MT638068.1 (449/449 bp), RPB2-MK606410.1 (861/861 bp), LSU-
218   MT533257.1 (726/726 bp), and the pathogen was identified as *Fusarium solani*. Sequence data
219   of TS2 showed homology to *Fusarium graminearum* (GenBank accessions ITS-KU254606.1
220   (789/789 bp), RPB2-LT222053.1 (970/976 bp), LSU-MH877271.1 (857/857 bp), and the
221   pathogen was identified as *Fusarium graminearum*.

Pathogenicity test of the pathogens

223   TS1 and TS2 show pathogenic effects on *D. officinale* in medium (Figure S1) and pot
224   conditions. The pathogen-incubated groups showed root rot symptoms after two weeks (Fig. 6),
225   while the roots of the control group were healthy and strong. TS1 was strongly pathogenic; most
226   seedling's root turned yellow and withered while the survival rate of the TS2 group was 55%,
227   and the roots turned yellow and black (Fig. 6 B, C).

Competitiveness of the four mycorrhizal fungi with pathogenic fungi

229   To test the interaction of four mycorrhizal fungi and pathogens, these strains were used to
230   confront these pathogens *in vitro*. All the pathogenic fungi grew with mycorrhizal fungi on the
231   medium, but the pathogens had a faster growth rate. The growth of TS1 was significantly
232   delayed under the effect of mycorrhizal fungi (Fig. 7 A-D). The growth curve indicated that the

233 growth rate of TS1 was obviously slowed down in 3-5 days when it came in close proximity to
234 the mycorrhizal fungus. The inhibition of mycorrhizal fungi on TS2 was not as obvious as that on
235 TS1, in which colonies touched each other (Fig. 7 E-H).

236 ## Mycorrhizal fungi improve root rot resistance of *D. officinale*

237 The competitive effect between the mycorrhizal fungi and pathogens *in vitro* did not
238 completely support the biocontrol potential of the mycorrhizal fungi in the hosts. Thus, we
239 evaluated the potential of mycorrhizal fungi as biocontrol agents against pathogens in *D.*
240 *officinale* on DE medium and in pots in greenhouses via interactions among hosts, mycorrhizal
241 fungi, and pathogens together. The survival rate of *D. officinale* indicated that four mycorrhizal
242 fungi played roles in improving the survival ability of tissue culture seedlings (Fig. 8). The
243 highest survival rate (~50% in TS1 and ~80% in TS2) indicated that MLX102 supplied stronger
244 disease resistance for *D. officinale*. The mycorrhizal fungus increased the survival rate to a
245 limited extent (0 ~ 20%) due to the weaker lethality of TS2 (Fig. 8A). The root rot rate and fresh
246 weight of *D. officinale* in the greenhouse both indicated improved disease resistance to
247 pathogenic fungal infection. The root rot rate of the CK groups (> 90%, Fig. 8 B) indicated that
248 both pathogenic fungi were highly virulent. MLX102 inhibited the root rot rate caused by TS1
249 and TS2 with an efficiency of approximately 40% ~ 50% (Fig. 8). GS222 seemed unable to
250 inhibit root rot caused by TS1, but it had a certain effect on TS2, with an inhibition rate of
251 approximately 25%. The other two mycorrhizal fungi had more moderate inhibition effects, with
252 inhibition efficiencies between those of MLX102 and GS222. All the mycorrhizal fungi led to an
253 increase in fresh weight to some extent. Although the reduction in root rot rate did not
254 correspond to an increase in fresh weight, all the different mycorrhizal fungi have the effect of
255 reducing the root rot rate and increasing the fresh weight (Fig. 8 C).
256

257 # Discussion

258 Mycorrhizal fungi are important resources for *D. officinale* growth promotion and intensify
259 their tolerance to the environment [43], with promising agricultural applications [44, 45]. But the
260 degree of specificity of most orchids to their mycorrhizal associates remains unknown. In this
261 study, we have shown that mycorrhizal fungi of *Cymbidium* could promote the growth of *D.*
262 *officinale* tissue culture seedlings and pot cultivation seedlings. Interestingly, mycorrhizal fungi
263 improved the adaptation of *D. officinale* to biotic stress and abiotic stress resistance. It is
264 important to note that the mycorrhizae from *Cymbidium* are compatible with *D. officinale*.
265 Literatures have shown that mycorrhizal fungi can promote the growth of orchids as well as
266 stimulation of germination [46-48]. However, few studies employed orchid-mycorrhizal fungi to
267 promote the growth of *D. officinale*. Moreover, we also found and identified the root rot pathogen
268 (soil-borne) of *D. officinale*s and mycorrhizal fungi could increase such biotic stress tolerance.
269 This finding will improve our understanding of the promotion effects of OMF on *D. officinale.*
270 According to reports, OMF have contributed to the growth, development, nutrient uptake,
271 and resistance to pathogenic infection of different plant species. These four fungi have been
272 proven that OMF could promote growth of orchids such as *C. hybrid*, *C. goeringii, C. mastersii* in
273 former studies [28-30]. In this study, we were able to prove that OMF significantly contributed to
274 both physiological and morphological parameters of *D. officinale*s expressing different growth
275 effect on *D. officinale.* The stem length, diameter and polysaccharide levels all increased,
276 indicating that the quality of the seedlings was improved.

277 The biochemical index quantificationally also demonstrated the promotion effect of these
278 fungi. Our results show that all four mycorrhizal fungi could increase the weight as well as
279 improve the content of photopigments (chlorophyll a, chlorophyll b and total chlorophyll), on
280 average. The increase of chlorophyll in the symbiotic plant probably resulted in higher
281 photosynthetic rates and thus improved plant biomass. The chlorophyll b content of *D. officinale*
282 in the medium was especially obvious. As an index of blue and violet light absorption, the high
283 chlorophyll b content indicated that mycorrhizal fungi could promote low light capacity utilization
284 [49]. This increase of chlorophyll content by mycorrhizal fungi inoculation may be due to an
285 increase in stomatal conductance, photosynthesis, transpiration [50, 51].
286 AcPase activity describes the phosphorus usage efficiency of plants. The decreasing
287 phosphatase efficiency in the mycorrhizal fungi coculture groups indicated that adequate
288 phosphorus was supplied. The OMF supplied the orchid with phosphorus (P) demonstrating that
289 the OMF was involved in P uptake and transfer in former studies [52, 53], which could provide
290 additional phosphorus to P acquired by the root of host plant. Similar studies of OMF proves
291 that P absorption and transfer abilities could provide phosphorus for host plant usage [54, 55].
292 Furthermore, difference in ACPase activity could be attributed to the metabolism of plant host
293 during interaction with OMF where the cell of OMF is digested in cavity and the activity occurs
294 with some level of variation [31, 56]. In addition, the AcPase activity of root could be different in
295 root after different mycorrhizal fungi species invade the host plant [57].
296 OMF could enhance the absorption ability of the root system as well as the hydraulic
297 conductivity of plants, which is associated with drought tolerance [58, 59].The increased survival
298 rate and decreased MDA content under drought conditions indicated that OMF increased the
299 drought tolerance of *D. officinale*. The potential of the mycorrhizal fungi to confer drought
300 resistance to host plants is important for plant adaptation to the environment [60, 61].
301 Root rot caused by pathogenic fungi has led to great economic losses in the production of
302 *D. officinale* and orchids [23, 62, 63]. Two pathogenic fungi were confirmed: TS1 and TS2 were
303 *Fusarium solani* and *Fusarium graminearum,* respectively, while the severe root rot in crops and
304 fruits could be caused by these fungi [23, 62, 64-68]. *Fusarium* species are one of the most
305 common soil-borne pathogens for plants and are causal agents of orchid root rot [69, 70]. These
306 pathogens are major problems for commercial orchid production [69, 71].
307 These pathogens also caused root rot in *D. officinale* in this study. TS1 is a highly
308 pathogenic fungus that causes a high lethal ratio under sterile medium conditions. However, the
309 seedling roots tended to rot in the pot after being inoculated with this pathogen. This pathogen
310 is relatively more likely to cause a high rate of root rot in greenhouses. This difference may be
311 because the DE medium is also suitable for fungal growth. It provides fungi with adequate
312 nutrients, which means that it helps pathogen growth and exerts virulence [72, 73]. The four
313 mycorrhizal fungi in this study exhibited resistance to pathogens on plates and pots, which was
314 mainly manifested both in the increased survival rate of seedlings and in reduced root rot of the
315 plants. They exhibited different inhibition zone diameters in the plate competition test, which
316 may be caused by antibiotics and lytic enzymes produced by the fungi that has reported in other
317 studies of mycorrhizal fungi [74-76]. These effects of the mycorrhizal fungi of *D. officinale* are
318 essential to the resistance of root rot pathogenic fungi in production.
319 Biological control has advantages, such as relatively few side effects, less pollution and
320 longer effective times, and is a major trend in the development of agriculture and forestry today

321   [44, 77]. This is an effective biocontrol method that is widely used in farming [78-80], and it is
322   also suitable for use in the production of *D. officinale* today. Mycorrhizal fungi could be important
323   biocontrol resources for competing with and inhibiting the reproduction of the pathogens by
324   producing antibiotic or antifungal compounds and stimulating plant defence responses [81, 82].
325   Abiotic stress could weaken plant defences and enhance pathogen infection probability [83]. It
326   has been suggested that the combination of abiotic and biotic stress could cause increased
327   serious disease [84, 85]. Mycorrhizal fungi could enhance resistance to both stresses,
328   indicating that these fungi are useful resources for the growth promotion of *D. officinale*.

329   ## Conclusions

330   Together, the mycorrhizal fungi in the culture medium or the cultivated substrate could
331   promote the production of *D. officinale* by increasing resistance to biological and abiotic stress.
332   Furthermore, we isolated and characterized two fungal root pathogens of *D. officinale*. Inhibition
333   of fungal plant pathogens by mycorrhizal fungi isolated from different *Cymbidium* was a suitable
334   means to control the disease caused by *Fusarium*. This finding is important for growth
335   promotion, root rot disease prevention and control in *D. officinale* production.

336

References

337 **References**

338 1. Zhao, Y., L. Xu, and Y. Hua, Effects of *Dendrobium officinale* polysaccharide on
339 adipogenic differentiation of rat bone marrow mesenchymal stem cells. Food Science and
340 Technology, 2017. **37**(2): p. 303-307.

341 2. Liu, X.-F., J. Zhu, S.-Y. Ge, L.-J. Xia, H.-Y. Yang, Y.-T. Qian, and F.-Z. Ren, Orally
342 administered *Dendrobium officinale* and its polysaccharides enhance immune functions in
343 BALB/c mice. Natural product communications, 2011. **6**(6): p. 867-870.

344 3. Xiang, L., C.S. Sze, T. Ng, Y. Tong, P. Shaw, C.S. Tang, and Y.K. Zhang, Polysaccharides
345 of *Dendrobium officinale* inhibit TNF-α-induced apoptosis in A-253 cell line. Inflammation
346 Research, 2013. **62**(3): p. 313-324.

347 4. Ng, T.B., J. Liu, J.H. Wong, X. Ye, S.C.W. Sze, Y. Tong, and K.Y. Zhang, Review of
348 research on *Dendrobium*, a prized folk medicine. Applied microbiology and biotechnology, 2012.
349 **93**(5): p. 1795-1803.

350 5. Tang, H., T. Zhao, Y. Sheng, T. Zheng, L. Fu, and Y. Zhang, *Dendrobium officinale* Kimura
351 et Migo: A Review on Its Ethnopharmacology, Phytochemistry, Pharmacology, and
352 Industrialization. Evid Based Complement Alternat Med, 2017: p. 7436259.

353 6. Lin, Y., J. Li, B. Li, T. He, and Z. Chun, Effects of light quality on growth and development
354 of protocorm-like bodies of *Dendrobium officinale* in vitro. Plant Cell, Tissue and Organ Culture
355 (PCTOC), 2011. **105**(3): p. 329-335.

356 7. Zhou, H., Q. Gao, Q. Gong, S. Qiu, J. He, J. Huang, and Y. He, First Report of Stem and
357 Root Rot of the Medicinal Herb *Dendrobium officinale* (Orchidaceae) Caused by *Ceratobasidium*
358 sp. AG-R in Guangxi, China. Plant Disease, 2017. **101**(9): p. 1679.

359 8. Otero, J.T., J.D. Ackerman, and P. Bayman, Diversity and host specificity of endophytic
360 Rhizoctonia-like fungi from tropical orchids. American Journal of Botany, 2002. **89**(11): p. 1852-
361 1858.

362 9. Tejesvi, M., M. Nalini, B. Mahesh, H. Prakash, K. Kini, H. Shetty, and V. Subbiah, New
363 hopes from endophytic fungal secondary metabolites. Bol Soc Quím Méx, 2007. **1**(1): p. 19-26.

364 10. Dearnaley, J.D.W., F. Martos, and M.-A. Selosse, Orchid Mycorrhizas: Molecular
365 Ecology, Physiology, Evolution and Conservation Aspects, in *Fungal Associations*, B. Hock,
366 Editor. 2012, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 207-230.

367 11. Dressler, R. and H. Rasmussen, Terrestrial Orchids: From Seed to Mycotrophic Plant.
368 Systematic Botany, 1996. **21**: p. 625.

369 12. Bidartondo, M.I., The evolutionary ecology of myco-heterotrophy. New phytologist,
370 2005. **167**(2): p. 335-352.

371 13. Aly, A.H., A. Debbab, and P. Proksch, Fungal endophytes: unique plant inhabitants with
372 great promises. Applied microbiology and biotechnology, 2011. **90**(6): p. 1829-1845.

373 14. Aly, A.H., A. Debbab, J. Kjer, and P. Proksch, Fungal endophytes from higher plants: a
374 prolific source of phytochemicals and other bioactive natural products. Fungal diversity, 2010.
375 **41**(1): p. 1-16.

376 15. McCormick, M., D. Whigham, D. Sloan, and K. O'Malley, Orchid-fungus fidelity: A
377 marriage meant to last? Ecology, 2006. **87**: p. 903-11.

378 16. Girlanda, M., R. Segreto, D. Cafasso, H.T. Liebel, M. Rodda, E. Ercole, S. Cozzolino, G.
379 Gebauer, and S. Perotto, Mediterranean meadow photosynthetic orchids feature partial myco-
380 eterotrophy and specific mycorrhizal associations. American journal of botany, 2011. **98**: p. 1148-
381 1163.

382 17. Xing, X., X. Ma, Z. Deng, J. Chen, F. Wu, and S. Guo, Specificity and preference of

383 mycorrhizal associations in two species of the genus *Dendrobium* (Orchidaceae). Mycorrhiza,
384 2013. **23**(4): p. 317-324.
385   18. Klironomos, J.N., Variation in plant response to native and exotic arbuscular mycorrhizal
386 fungi. Ecology, 2003. **84**(9): p. 2292-2301.
387   19. Raju, P., R. Clark, J. Ellis, and J. Maranville, Effects of species of VA-mycorrhizal fungi
388 on growth and mineral uptake of sorghum at different temperatures. Plant and soil, 1990. **121**(2):
389 p. 165-170.
390   20. Kosuta, S., M. Chabaud, G. Lougnon, C. Gough, J. Dénarié, D.G. Barker, and G. Bécard,
391 A Diffusible Factor from Arbuscular Mycorrhizal Fungi Induces Symbiosis-Specific MtENOD11
392 Expression in Roots of *Medicago truncatula.* Plant physiology, 2003. **131**(3): p. 952-962.
393   21. Jacquemyn, H., K.J. Duffy, and M.-A. Selosse, Biogeography of orchid mycorrhizas, in
394 *Biogeography of mycorrhizal symbiosis*. 2017, Springer. p. 159-177.
395   22. Hossain, M.M., R. Kant, P.T. Van, B. Winarto, S. Zeng, and J.A. Teixeira da Silva, The
396 application of biotechnology to orchids. Critical Reviews in Plant Sciences, 2013. **32**(2): p. 69-
397 139.
398   23. Bodah, E.T., Root rot diseases in plants: a review of common causal agents and
399 management strategies. Agri Res & Tech: Open Access J, 2017. **5**(3): p. 555661.
400   24. Zhang, Y., B. Lin, M. Zou, J. Liang, and H. Hu, First Report of *Fusarium* Wilt of
401 *Dendrobium officinale* Caused by *Fusarium oxysporum* in China. Plant Disease, 2017. **101**(6): p.
402 1039-1039.
403   25. Sardrood, B.P. and E.M. Goltapeh, Effect of Agricultural Chemicals and Organic
404 amendments on biological control fungi, in *Sustainable Agriculture Reviews 31*. 2018, Springer.
405 p. 217-359.
406   26. Mahanty, T., S. Bhattacharjee, M. Goswami, P. Bhattacharyya, B. Das, A. Ghosh, and P.
407 Tribedi, Biofertilizers: a potential approach for sustainable agriculture development.
408 Environmental Science and Pollution Research, 2017. **24**(4): p. 3315-3335.
409   27. Singh, J.S., V.C. Pandey, and D. Singh, Efficient soil microorganisms: a new dimension
410 for sustainable agriculture and environmental development. Agriculture, ecosystems &
411 environment, 2011. **140**(3-4): p. 339-353.
412   28. Guo, S.T., W.U. Jian-Rong, H.U. Jun, H.G. Yang, L.U. Lu, and F. Liu, Symbiotic seed
413 germination of *Cymbidium mastersii* Griff.ex Lindl. Journal of Yunnan University(Natural
414 Sciences Edition), 2012. **34**(3): p. 348-355.
415   29. Wu, J., H. Ma, X. Xu, N. Qiao, S. Guo, F. Liu, D. Zhang, and L. Zhou, Mycorrhizas alter
416 nitrogen acquisition by the terrestrial orchid *Cymbidium goeringii.* Annals of Botany, 2013(6): p.
417 1181-1187.
418   30. Dong, F., Screen several orchid mycorrhizal fungi and preliminary test on the seed
419 germination condition. 2008, Beijing Forestry University.
420   31. Jin, H., Z.X. Xu, H. Chen, and S.F. Han, Localization and changes of activity of acid
421 phosphatase in mycorhiza of *Cymbidium hookerianum.* Journal of Beijing Forestry University,
422 2007. **29**(4): p. 156-160.
423   32. Dijk, E. and N.D. Eck, Effects of mycorrhizal fungi on in vitro nitrogen response of some
424 Dutch indigenous orchid species. Canadian Journal of Botany, 1995. **73**(8): p. 1203-1211.
425   33. Murashige, T. and F. Skoog, A revised medium for rapid growth and bioassay with
426 tobacco tissue cultures. Physiologia Plantarum, 1962. **15**(3): p. 473-497.
427   34. Gilbertson, R.L., The use of dry-leaf inoculum for establishment of common bacterial
428 blight of beans. Plant Disease, 1988. **72**(5): p. 385-389.

429   35. Munro, R.C., J. Wilson, J. Jefwa, and K.W. Mbuthia, A low-cost method of mycorrhizal
430   inoculation improves growth of *Acacia tortilis* seedlings in the nursery. Forest Ecology and
431   Management, 1999. **113**(1): p. 51-56.

432   36. Şükran, D., T. GÜNEŞ, and R. Sivaci, Spectrophotometric determination of chlorophyll-
433   A, B and total carotenoid contents of some algae species using different solvents. Turkish Journal
434   of Botany, 1998. **22**(1): p. 13-18.

435   37. White, T., T. Bruns, S. Lee, F. Taylor, T. White, S.H. Lee, L. Taylor, and J. Shawetaylor,
436   Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR
437   protocols: a guide to methods and applications. 1990: p. 315-322.

438   38. Phylogenetic Diversity and Microsphere Array-Based Genotyping of Human Pathogenic
439   Fusaria, Including Isolates from the Multistate Contact Lens-Associated U.S. Keratitis Outbreaks
440   of 2005 and 2006. Journal of Clinical Microbiology, 2007. **45**(7): p. 2235-2248.

441   39. A, G.H.S., J.M.S. B, H.J. C, and J.W.S. A, A multi-gene phylogeny of *Clavicipitaceae*
442   (*Ascomycota*, Fungi): identification of localized incongruence using a combinational bootstrap
443   approach. Molecular Phylogenetics & Evolution, 2007. **44**(3): p. 1204-1223.

444   40. Meyer, M. and M.K. Hausbeck, Using Soil-Applied Fungicides to Manage Phytophthora
445   Crown and Root Rot on Summer Squash. Plant Disease, 2013. **97**(1): p. 107-112.

446   41. Dubey, M.K., A. Broberg, F.J. Dan, and M. Karlsson, Role of the methylcitrate cycle in
447   growth, antagonism and induction of systemic defence responses in the fungal biocontrol agent
448   *Trichoderma atroviride.* Microbiology-sgm, 2013. **159**(Pt_12): p. 2492-2500.

449   42. Dubey, M.K., F.J. Dan, and M. Karlsson, An ATP-binding cassette pleiotropic drug
450   transporter protein is required for xenobiotic tolerance and antagonism in the fungal biocontrol
451   agent *Clonostachys rosea.* Mol Plant Microbe Interact, 2014. **27**(7).

452   43. Chen, J., H. Wang, and S.X. Guo, Isolation and identification of endophytic and
453   mycorrhizal fungi from seeds and roots of *Dendrobium* (Orchidaceae). Mycorrhiza, 2012. **22**(4):
454   p. 297-307.

455   44. Vega, F.E., The use of fungal entomopathogens as endophytes in biological control: a
456   review. Mycologia, 2018. **110**(1): p. 4-30.

457   45. Shahzad, R., A.L. Khan, S. Bilal, S. Asaf, and I.-J. Lee, What is there in seeds? Vertically
458   transmitted endophytic resources for sustainable improvement in plant growth. Frontiers in plant
459   science, 2018. **9**: p. 24.

460   46. Brundrett and C. Mark, Scientific approaches to Australian temperate terrestrial orchid
461   conservation. Australian Journal of Botany, 2007. **55**(3): p. 293-307.

462   47. Jacquemyn, H., M. Waud, V. Merckx, B. Lievens, and R. Brys, Mycorrhizal diversity,
463   seed germination and long-term changes in population size across nine populations of the
464   terrestrial orchid *Neottia ovata.* Molecular Ecology, 2015. **24**(13): p. 3269-3280.

465   48. Zi, X.M., C.L. Sheng, U.M. Goodale, S.C. Shao, and J.Y. Gao, In situ seed baiting to
466   isolate germination-enhancing fungi for an epiphytic orchid, *Dendrobium aphyllum*
467   (Orchidaceae). Mycorrhiza, 2014. **24**(7): p. 487-499.

468   49. Ohashi-Kaneko, K., K. Fujiwara, Y. Kimura, and K. Kurata, Effect of Blue-Light PPFD
469   Percentage in Red and Blue LED Low-Light Irradiation during Storage on the Contents of
470   Chlorophyll and Rubisco in Grafted Tomato Plug Seedlings. Environment Control in Biology,
471   2010. **44**(4): p. 309-314.

472   50. Kirschner, R., Response of Arbuscular Mycorrhizal Fungi on Growth and Chlorophyll
473   Content of Three Varieties of *Gossypium herbaceum* L. Plant Pathology & Quarantine, 2013. **3**:
474   p. 54-57.

475   51. Rajasekaran, S., S.M. Nagarajan, K. Arumugam, R. Saravanamuthu, and S. Balamurugan,
476   Effect of dual inoculation (Rhizobium and AM fungi) on chlorophyll content of *Arachis hypogaea*
477   L. CV. TMV-2. Plant Archives, 2006. **6**(2): p. 671-672.

478   52. Cameron, D., J. Irene, J.R. Leake, and D.J. Read, Mycorrhizal Acquisition of Inorganic
479   Phosphorus by the Green-leaved Terrestrial Orchid *Goodyera repens.* Ann Bot, 2007(5): p. 831-
480   834.

481   53. Alexander, C. and I. Hadley, Phosphate uptake by in relation to mycorrhizal infection.
482   New Phytologist, 1984. **97**(3): p. 401-411.

483   54. Plassard, C., A. Becquer, and K. Garcia, Phosphorus transport in mycorrhiza: how far are
484   we? Trends in plant science, 2019. **24**(9): p. 794-801.

485   55. Siti, N., N.D. Swarts, K.W. Dixon, L. Hans, and D.J. Merritt, Variation in nutrient-
486   acquisition patterns by mycorrhizal fungi of rare and common orchids explains diversification in
487   a global biodiversity hotspot. Annals of Botany, 2013(6): p. 1233-1241.

488   56. Liu, C.Y., The changes of the vitality of *Armillaria mellea* and the histochemical
489   localization of some enzymes in the hyphae digested period of *gastrodia elata.* Acta Botanica
490   Sinica, 1982. **24**(4): p. 307-311.

491   57. Conn, C. and J. Dighton, Litter quality influences on decomposition, ectomycorrhizal
492   community structure and mycorrhizal root surface acid phosphatase activity. Soil Biology &
493   Biochemistry, 2000. **32**(4): p. 489-496.

494   58. Zaidi, A., M.S. Khan, M. Ahemad, M. Oves, and P. Wani, Recent advances in plant
495   growth promotion by phosphate-solubilizing microbes. Microbial strategies for crop improvement,
496   2009: p. 23-50.

497   59. Smith, S.E., E. Facelli, S. Pope, and F.A. Smith, Plant performance in stressful
498   environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas.
499   Plant and Soil, 2010. **326**(1-2): p. 3-20.

500   60. Singh, L.P., S.S. Gill, and N. Tuteja, Unraveling the role of fungal symbionts in plant
501   abiotic stress tolerance. Plant signaling & behavior, 2011. **6**(2): p. 175-191.

502   61. Rapparini, F. and J. Peñuelas, Mycorrhizal fungi to alleviate drought stress on plant
503   growth, in *Use of Microbes for the Alleviation of Soil Stresses, Volume 1*. 2014, Springer. p. 21-
504   42.

505   62. Wang, R.Y., B. Gao, X.H. Li, J. Ma, and S.L. Chen, First Report of *Fusarium solani*
506   Causing *Fusarium* Root Rot and Stem Canker on Storage Roots of Sweet Potato in China. Plant
507   Disease, 2014. **98**(1): p. 160-160.

508   63. Latiffah, Z., M.Z.N. Hayati, S. Baharuddin, and Z. Maziah, Identification and
509   Pathogenicity of *Fusarium* Species Associated with Root Rot and Stem Rot of *Dendrobium.* Asian
510   Journal of Plant Pathology, 2010. **3**(1): p. 14-21.

511   64. Mesterhazy, A., M. Lemmens, and L.M. Reid, Breeding for resistance to ear rots caused
512   by *Fusarium* spp. in maize–a review. Plant Breeding, 2012. **131**(1): p. 1-19.

513   65. Nemec, S. and R.M. Zablotowicz, Effect of soil temperature on root rot of rough lemon
514   caused by *Fusarium solani.* Mycopathologia, 1981. **76**(3): p. 185-190.

515   66. Bohra, B. and K. Mathur, Biocontrol Agents and Neem Formulations for Suppression of
516   *Fusarium solani* Root Rot in Soybean. Journal of Mycology & Plant Pathology, 2004. **34**(2): p.
517   408-409.

518   67. Lee, T.V.D., H. Zhang, A.V. Diepeningen, and C. Waalwijk, Biogeography of *Fusarium
519   graminearum* species complex and chemotypes: a review. Food Additives & Contaminants: Part
520   A, 2015. **32**(4): p. 453-460.

521 68. Kikot, G.E., R.A. Hours, and T.M. Alconada, Contribution of cell wall degrading enzymes
522 to pathogenesis of *Fusarium graminearum*: a review. Journal of Basic Microbiology, 2009. **49**(3):
523 p. 231-241.
524 69. Swett, C.S. and J.Y. Uchida, Characterization of Fusarium diseases on commercially
525 grown orchids in Hawaii. Plant Pathology, 2015. **64**(3): p. 648-654.
526 70. Latiffah, N. Hayati, Z. M., Baharuddin, and Maziah, Vegetative Compatibility Group of
527 Fusarium species Associated with Root and Stem Rot of Orchid. Malaysian Journal of
528 Microbiology, 2008. **4**(1): p. 49-52.
529 71. Srivastava, S., Characterization and management of different *Fusarium* species associated
530 with orchids cultivated in Hawai'i. Dissertations & Theses - Gradworks, 2014. **76**(01): p. 131.
531 72. Lu, Y., C. Su, N.V. Solis, S.G. Filler, and H. Liu, Synergistic regulation of hyphal
532 elongation by hypoxia, CO2, and nutrient conditions controls the virulence of *Candida albicans*.
533 Cell host & microbe, 2013. **14**(5): p. 499-509.
534 73. Fernandez, J., M. Marroquin-Guzman, and R.A. Wilson, Mechanisms of nutrient
535 acquisition and utilization during fungal infections of leaves. Annual review of phytopathology,
536 2014. **52**: p. 155-174.
537 74. Wanderley, M., J.M.W.D. Neto, J. Filho, C. Lima, and A. Porto, Collagenolytic enzymes
538 produced by fungi: a systematic review. Brazilian Journal of Microbiology, 2017. **48**(1): p. 13-24.
539 75. Krywolap, G.N., L.F. Grand, and L. Jr, The natural occurrence of an antibiotic in the
540 mycorrhizal fungus *Cenococcum graniforme*. Canadian Journal of Microbiology, 2011. **10**(3): p.
541 323-328.
542 76. Santoro, T. and L.E. Casida, Elaboration of antibiotics by Boletus luteus and certain other
543 mycorrhizal fungi. Canadian Journal of Microbiology, 1962. **8**(1): p. 43-48.
544 77. O'Brien, P.A., Biological control of plant diseases. Australasian Plant Pathology, 2017.
545 **46**(4): p. 293-304.
546 78. Toghueo, R.M.K., P. Eke, Í. Zabalgogeazcoa, B.R.V. de Aldana, L.W. Nana, and F.F.
547 Boyom, Biocontrol and growth enhancement potential of two endophytic *Trichoderma* spp. from
548 *Terminalia catappa* against the causative agent of Common Bean Root Rot (*Fusarium solani*).
549 Biological Control, 2016. **96**: p. 8-20.
550 79. Egamberdieva, D., S.J. Wirth, V.V. Shurigin, A. Hashem, and E.F. Abd_Allah,
551 Endophytic bacteria improve plant growth, symbiotic performance of chickpea (*Cicer arietinum*
552 L.) and induce suppression of root rot caused by *Fusarium solani* under salt stress. Frontiers in
553 Microbiology, 2017. **8**: p. 1887.
554 80. Jaber, L.R. and B.H. Ownley, Can we use entomopathogenic fungi as endophytes for dual
555 biological control of insect pests and plant pathogens? Biological control, 2018. **116**: p. 36-45.
556 81. Dell, B., Role of mycorrhizal fungi in ecosystems. CMU. Journal, 2002. **1**(1): p. 47-60.
557 82. Gao, F., C. Dai, and X. Liu, Mechanisms of fungal endophytes in plant protection against
558 pathogens. Afr J Microbiol Res, 2010. **4**(13): p. 1346-1351.
559 83. Mittler, R. and E. Blumwald, Genetic Engineering for Modern Agriculture: Challenges
560 and Perspectives. Annual Review of Plant Biology, 2010. **63**(1): p. 443-462.
561 84. MacDonald, J., and D., Effect of Salinity Stress on the Development of Phytophthora Root
562 Rot of *Chrysanthemum*. Phytopathology, 1982. **72**(2): p. 214-214.
563 85. Pandey, P., R. Sinha, K.S. Mysore, and M. Senthil-Kumar, Impact of Concurrent Drought
564 Stress and Pathogen Infection on Plants, in *Combined Stresses in Plants: Physiological,*
565 *Molecular, and Biochemical Aspects*, R. Mahalingam, Editor. 2015, Springer International
566 Publishing: Cham. p. 203-222.

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Figure 1

Mycorrhizal fungi promote the growth of tissue culture seedlings of *Dendrobium officinale.*

GDB254, MLX102, GS222, and GDB162 were inoculated into *D. officinale* seedlings for 60 days and were associated with increased weight acquisition compared to that in the control group (CK).
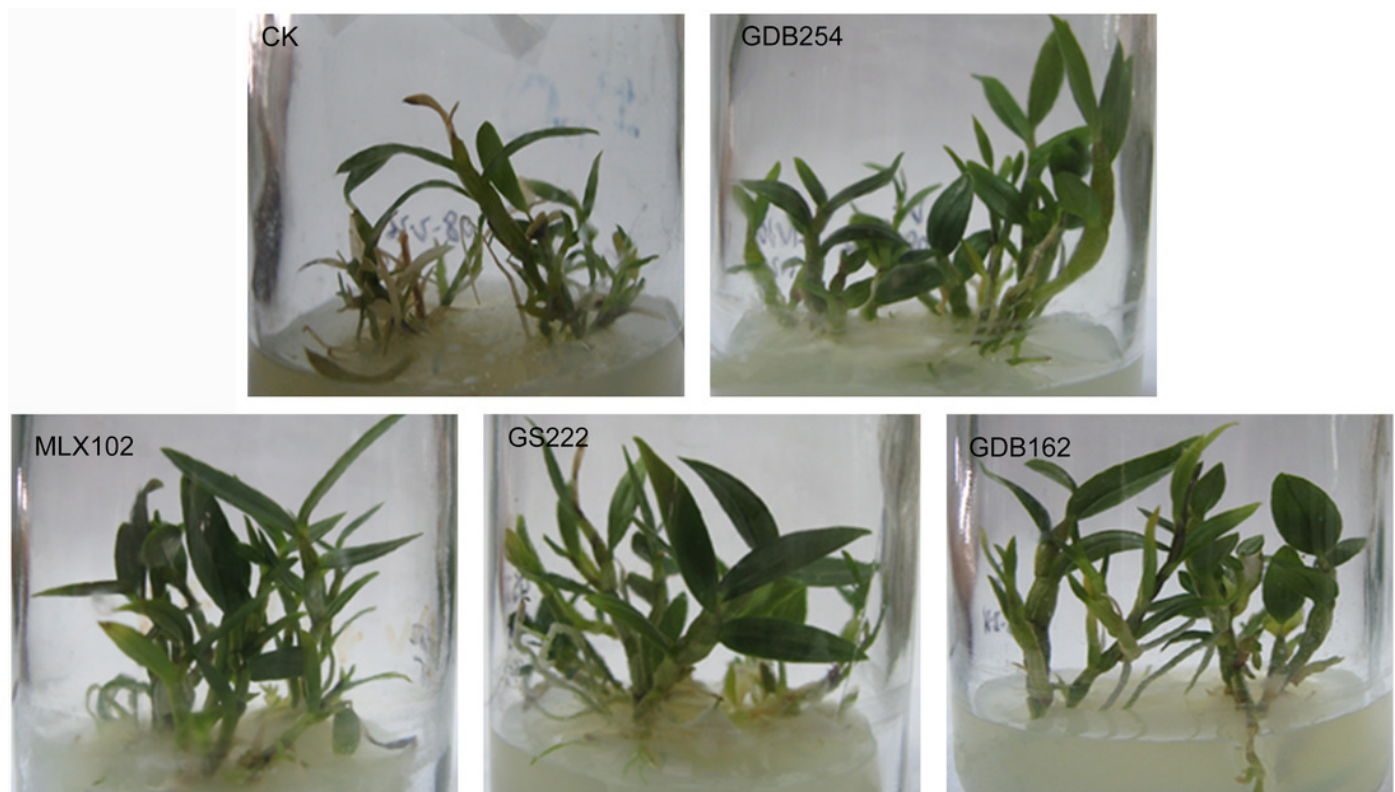
Figure 2

Mycorrhizal fungi promote the growth of tissue culture seedlings of *Dendrobium officinale.*

The growth-promoting effects of GDB254, MLX102, GS222, and GDB162 on *D. officinale* tissue culture seedlings after 60 days. (A), (B), (C), and (D) show the fresh weight, chlorophyll content, stem polysaccharide levels, and root acid phosphatase activity of *D. officinale*, respectively; the values of the parameters increased after symbiotic growth with different mycorrhizal fungi. Letters over the bars indicate significant differences at the 5% level.
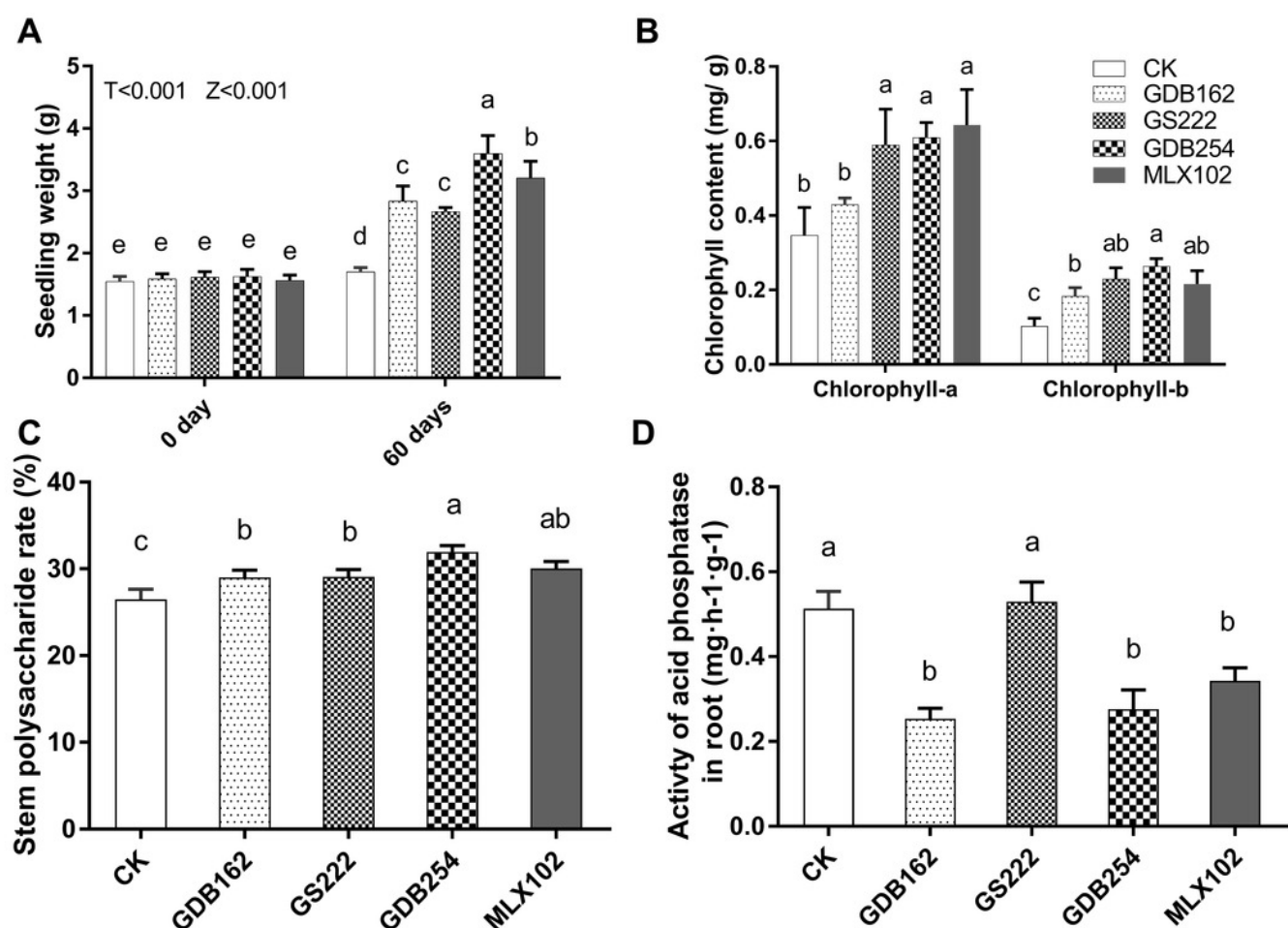
Figure 3

Growth-promoting effects of mycorrhizal fungi on *Dendrobium officinale* cultivated in pots.

(A) Effects of mycorrhizal fungi on the survival rate after transplantation from the culture bottles to flower pots. (B, C, and D) Growth-promoting effects of various mycorrhizal fungi on (B) the stem diameter, (C) stem length, and (D) leaf number of the plants grown in pots. Different letters over the bars indicate significant differences at the 5% level.
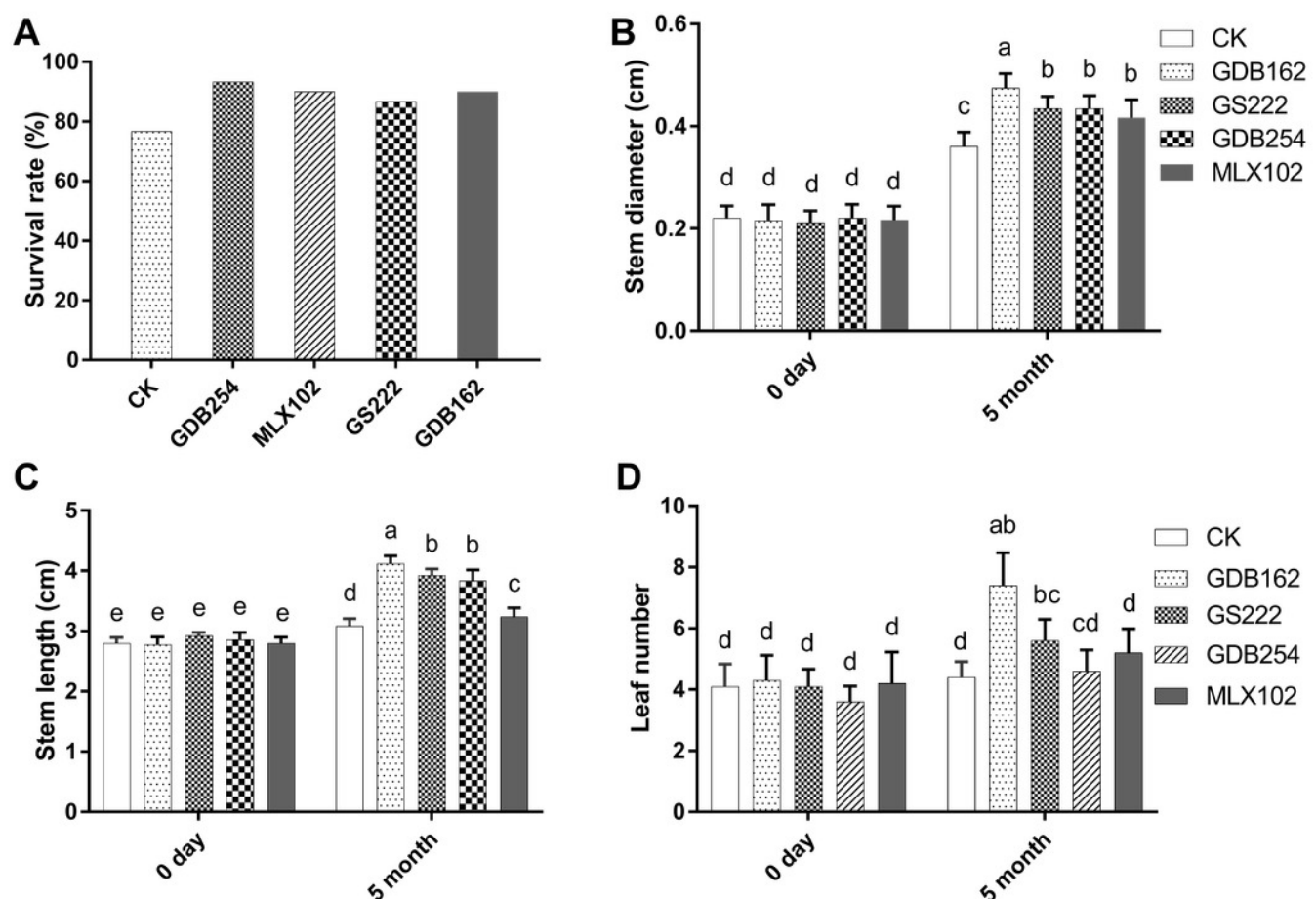
Figure 4

The effect of mycorrhizal fungi on the drought resistance of *D. officinale.*

(A) The effect of mycorrhizal fungi on the survival rate under drought conditions. (B) The effect of mycorrhizal fungi on the MDA contents of *D. officinale* under various drought conditions.
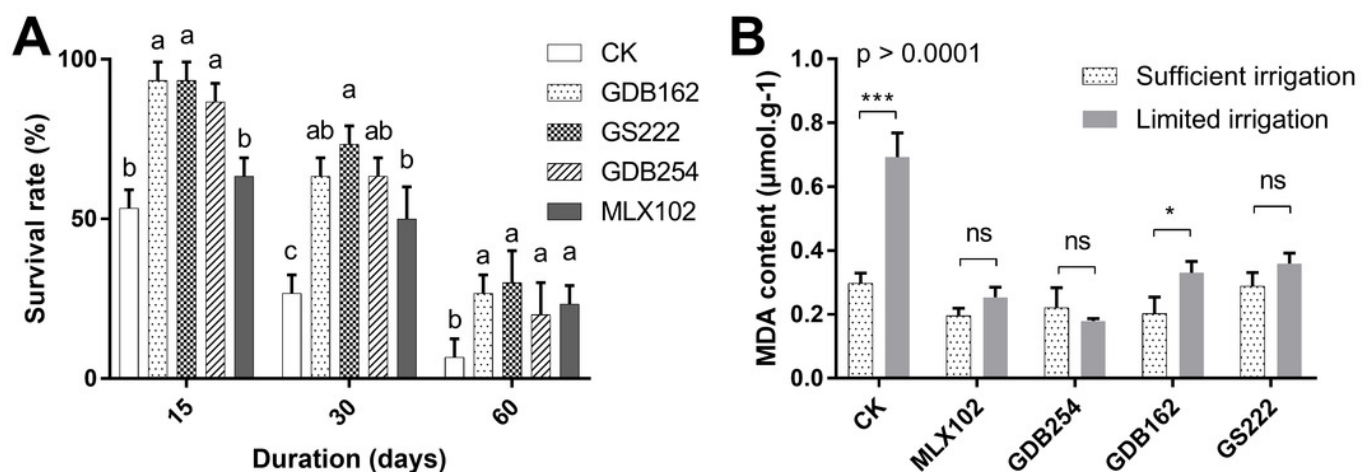
Figure 5

Morphology and microstructure of pathogenic fungi.

Morphology of TS1 (A) and TS2 (D) strains grown on PSA. (B), (C) are the microscopic morphology of TS1. (E), (F) are the microscopic morphology of TS2. Colonies of TS1 were formed after 3 days of growth on PSA medium at 28°C
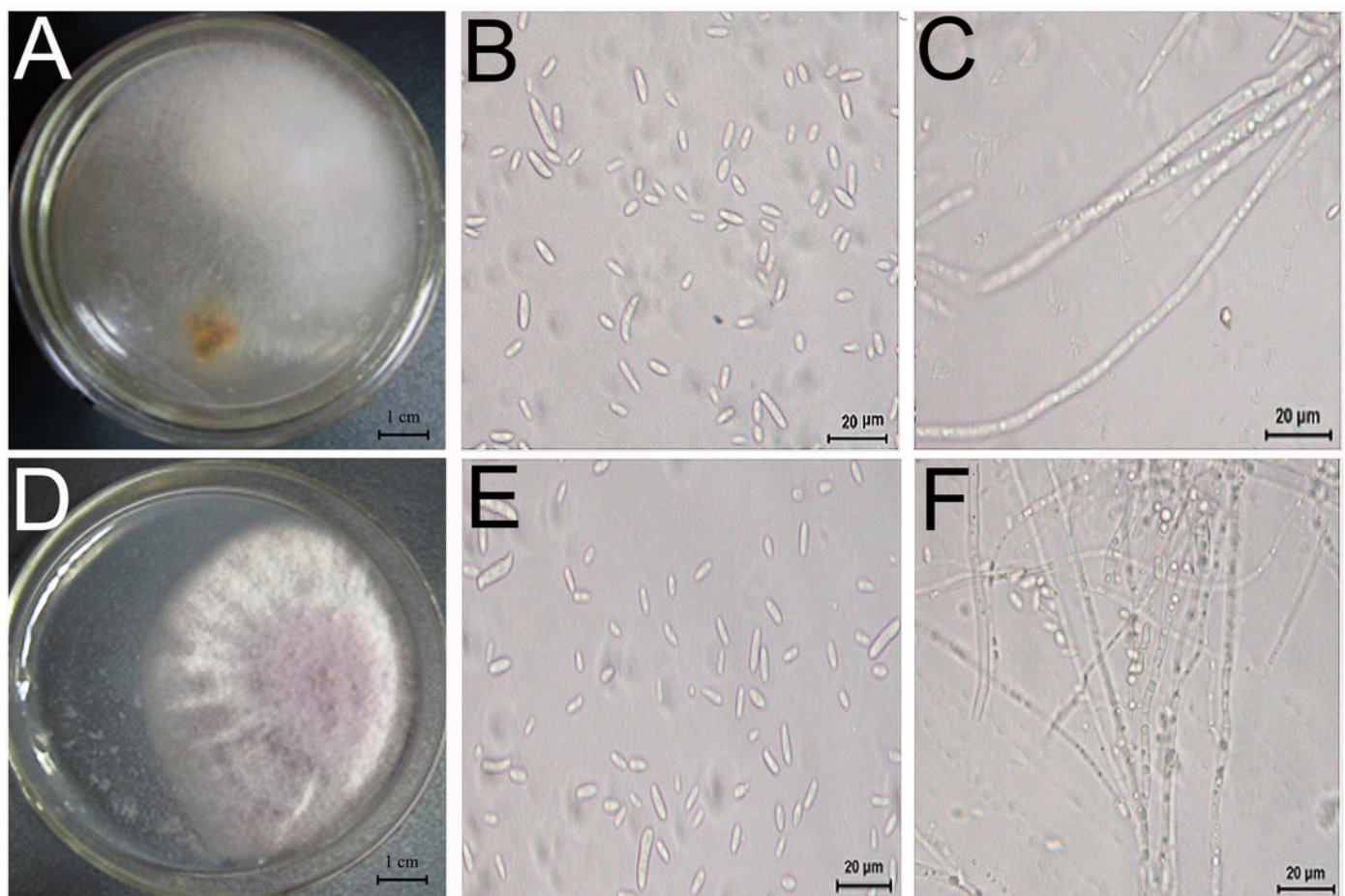
Figure 6

TS1 and TS2 strains all showed pathogenicity to *Dendrobium officinale.*

(A) is the control group, which was not inoculated with pathogenic fungus. TS1 (B) and TS2 (C) were incubated with *D. officinale* seedlings. The solid arrows point to the rot root and hollow arrow point to healthy root.
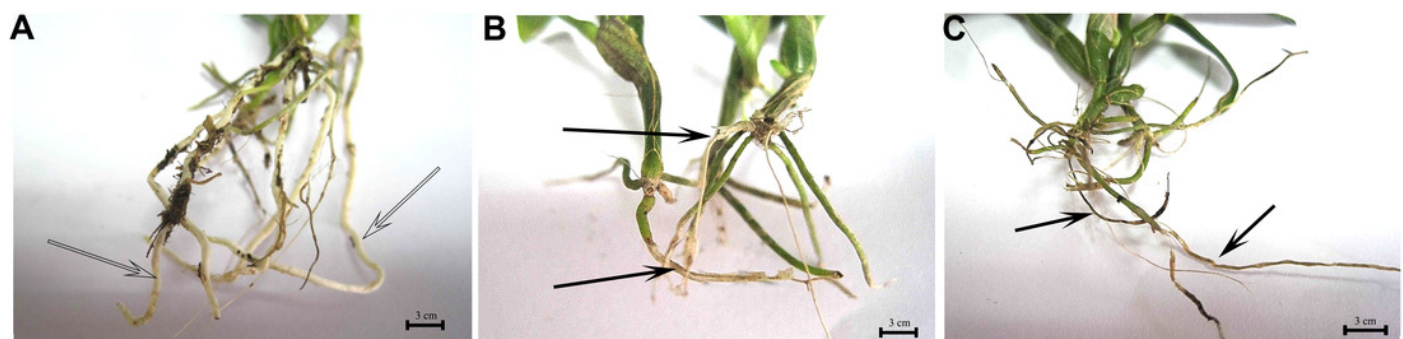
Figure 7

The competitive effect of mycorrhizal fungi with pathogenic fungi.

The mycorrhizal fungi (GDB254, GS222 MLX 102, and GDP162) competed with the pathogenic fungi (TS1 and TS2) by means of orthogonal experimental design, and the growth curves of the pathogenic fungi were plotted and analysed. (A-D) shows the mycorrhizal fungus competing with TS1 and (E-H) shows the mycorrhizal fungus competing with TS2.
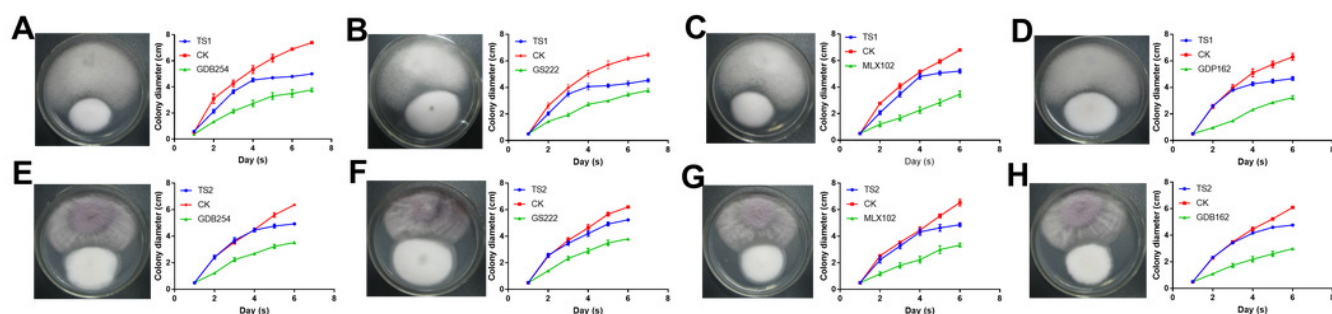
Figure 8

Mycorrhizal fungi increase the resistance of *Dendrobium officinale*.

Mycorrhizal fungi interacting with *D. officinale* could increase its disease resistance and thus improve its survival rate on DE medium (A), inhibit root rot (B) and increase fresh weight yield (C) under disease stress.