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Inoculation with *Glomus mosseae* Improves the Growth and Salvianolic Acid B Accumulation of Continuously Cropped *Salvia miltiorrhiza*

Meilan Chen [1,†], Guang Yang [1,†], Dahui Liu [2], Minhui Li [3], Hongyan Qiu [4], Lanping Guo [1], Luqi Huang [1,*] and Zhi Chao [5,*]

1 State Key Laboratory of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China; chenmeilan-99@163.com (M.C.); hbykdxyg2008@163.com (G.Y.); glp01@126.com (L.G.)
2 Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences, Kunming 650200, China; juhuacha2007@sohu.com
3 School of Pharmacy, Baotou Medical College, Baotou 014060, China; li_minhui@aliyun.com
4 School of Public Health, Ningxia Medical University, Yinchuan 750004, China; yanzide80@163.com
5 School of Traditional Chinese Medicine, Southern Medical University, Guangzhou 510515, China
* Correspondence: huangluqi01@126.com (L.H.); chaozhi@smu.edu.cn (Z.C.); Tel.: +86-10-6401-4411 (L.H.); +86-20-6164-8764 (Z.C.)
† These two authors contributed equally to this work.

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Abstract: *Salvia miltiorrhiza* (*S. miltiorrhiza*) Bunge is one of the most economically important medicinal crops in China. In traditional Chinese medicine, its root is used as an important ingredient in formulas for treatment of atherosclerosis-related disorders. The continuous cropping of *S. miltiorrhiza* increases the proportion of dried seedlings and decreases the biomass of the shoots and roots and the contents of active components. In this study, three field experiments were conducted to investigate the effects of *Glomus mosseae* (*G. mosseae*) inoculation on the growth and contents of active ingredients and nutrients in continuously cropped *S. miltiorrhiza*. The results showed that inoculation with *G. mosseae* increased the shoot biomass of *S. miltiorrhiza* by 48.1% and the root biomass by 39.2%, and decreased the dried seedling rate by nearly 75%. Inoculation with *G. mosseae* also increased the salvianolic acid B concentration by 21.9% in the shoots and 9.2% in the roots of *S. miltiorrhiza*, and also significantly increased Mn concentration in the roots and shoots (by 65.1% and 93.4%, respectively) and Fe concentration in the roots (by 75%). The accumulation of salvianolic acid B, Mn, and Fe in *G. mosseae* inoculated *S. miltiorrhiza* may be a mechanism that imparts tolerance to continuous cropping. Inoculation of *S. miltiorrhiza* with *G. mosseae* can serve as an effective approach of biocontrol to improve the performance of continuously cropped *S. miltiorrhiza*.

Keywords: *Salvia miltiorrhiza*; continuous cropping; arbuscular mycorrhizal fungus; *Glomus mosseae*; salvianolic acid B; growth

1. Introduction

Salvia miltiorrhiza Bunge is one of the most economically important medicinal crops in China. In traditional Chinese medicine, its roots (called 'Danshen' for its reddish-brown surface) are used for treatment of a variety of ailments including cerebrovascular and cardiovascular diseases [1], hypertension [2], ischemic stroke [3], breast cancer [4], and hepatitis [5]. Salvianolic acid B is one of the most abundant and bioactive compounds of Danshen [6], which has been suggested to exert various pharmacological effects, such as anti-oxidation, anti-inflammation and anti-tumor [7–9];

it has been clinically used in patients having coronary vascular diseases with successful results in China [10,11]. In China, *S. miltiorrhiza* is grown mostly by continuous cultivation, which has resulted in declined productivity and quality of the crop, a condition also seen in other crops such as maize, soybean, and wheat following years of continuous cultivation [12–14], and such decline has resulted in substantial agricultural and economic losses. A report showed that *S. miltiorrhiza* could tolerate a maximum of three years of continuous cropping; the annual yield and quality began to reduce significantly thereafter, and the harvest could be limited even in the third year of continuous cropping [15].

Continuous cropping of *S. miltiorrhiza* also causes soil deterioration [16] and results in an imbalance of nutrient availability and accumulation of soil-borne pathogens [17,18]. Currently three strategies have been devised to improve the soil quality following continuous cropping, namely physical remediation, chemical remediation, and bioremediation [19]. As a promising approach to soil bioremediation, mycorrhizal symbiosis has shown potential as an alternative to chemical fertilizer and pesticide use in sustainable agriculture [20]. The presence of arbuscular mycorrhizal fungi (AMF) in the rhizosphere soil has been shown to (1) significantly improve nutrient supply to the plants [21]; (2) provide protection of the roots against pathogens [22,23]; (3) improve the soil conditions for the host plants by improving the soil structure and soil aggregate stability [24,25]; and (4) contribute to the ecosystem stability by serving as a biofertilizer [26]. In addition, AMF can also improve the contents of secondary metabolites in the host plants [27,28].

Among the noticeable AMFs, *Glomus mosseae* (T. H. Nicolson & Gerd.) Gerd. & Trappe (=*Funneliformis mosseae*) is well-known for its benefits to many crops [29,30]. So far, the effects of *G. mossae* on the growth, active ingredients and nutrient contents of continuously cropped *S. miltiorrhiza* remain unknown. In this study, we carried out a field test to evaluate the beneficial effects of AMF on cropping of *S. miltiorrhiza* so as to provide evidence for utilization of AMF in *S. miltiorrhiza* cultivation.

2. Materials and Methods

2.1. Study Area

The field test was conducted in Laiwu station (36°20′ N, 117°41′ E), Shandong Province, China, which is a long-term experimental base for Danshen cultivation used by the China Academy of Chinese Medical Sciences. Laiwu City is located in a warm temperate semi-humid monsoon climate region (Figure 1). According to literature reports [31], during years 1957–2008, the average annual rainfall was 695.1 mm, and the average precipitation in summer was 454.2 mm, accounting for about 65.3% of annual precipitation; the average annual temperature was between 10.9 °C and 14.2 °C, with a mean of 13.3 °C; the average annual sunshine duration was about 1929 h.

Figure 1. Study site on the map.

The farm operations of this experiment were similar to those of local farmers and did not involve protected or endangered species. This experiment was approved by China Academy of Chinese Medical Sciences. The land had been continuously planted with *S. miltiorrhiza* for four years, and there was a high incidence of soil-borne diseases. The soil was classified as weathered rock (50% rock) with a pH value of 5.51 and contained 11.51% of organic carbon, 11.5 g/kg of organic material, 2.3 mg/kg of available phosphorus (P), 38 mg/kg of total P, 115 mg/kg of total nitrogen, 170 mg/kg of total potassium, 0.85 mg/kg of available zinc, 14.9 mg/kg of available manganese, 17.2 mg/kg of available iron, and 0.81 mg/kg of available copper.

2.2. Mycorrhizal Inocula

Mycorrhizal inocula were obtained from sand cultures of *Glomus mosseae*, which were originally provided by the Soil and Fertilizer Institute, Chinese Academy of Agricultural Sciences, Beijing, China. Clover (*Trifolium repens*) was used as the host, and the inocula contained sand, spores, hyphae, and clover root fragments.

2.3. Plant Material

A white-flower forma of *S. miltiorrhiza* native to Shangdong Province, i.e., *S. miltiorrhiza* f. *alba* [32] was used in the study. The forma yields Danshen crude drug of better quality [33]. The whole experiment was performed in the year of 2013. Sowing of seeds was carried out in mid-March. The seeds, collected at the cultivating base by the authors, were treated with 75% ethanol for 2 min and then with 30% hydrogen peroxide solution for 10 min, washed with tap water, immersed in water at room temperature for 8–12 h, and then sown in pots containing moist sterilized vermiculite. Before sowing, 10 g of AMF inocula—which contained approximately 500 spores—was embedded 2 cm below the soil surface. For non-mycorrhizal treatments, inocula that had been autoclaved at 121 °C for 20 min were used. The seeds were germinated at 25–28 °C. The control plants were treated in the same manner as the inoculated plants.

In late April, uniformly sized 50-day-old healthy seedlings were carefully uprooted to minimize the damage to the roots and transplanted in the field. In total, six plots, three for inoculated plants with the AMF *G. mosseae* and three for control plants without microbial inoculation, were employed. In each plot, which measured 4 × 4 m with four 4-m-long rows, a total of 100 seedlings were planted. The plots were arranged in randomized blocks. The plants were harvested six months after the transplantation.

2.4. Root Staining for Evaluation of AMF Root Colonization

At 50 days after inoculation, the presence of AMF in 10 of the inoculated *S. miltiorrhiza* plants was examined by root staining using the method of Biermann and Linderman with modifications [34]. Briefly, the live healthy roots were washed thoroughly in distilled water and cut into 0.5-cm-long segments. The roots were bleached in hot (90–100 °C) 10% KOH solution, rinsed three times with distilled water, and immersed in 10% HCl solution for 20 min before staining with 0.05% trypan blue in an acid glycerol solution at 90 °C to 100 °C for 3 min. The samples of the roots were observed under a light microscope (Nikon Eclipse 80i, Tokyo, Japan).

2.5. Assessment of Plant Disease and Growth

From each plot, all the 100 plants were assessed of plant disease by evaluating their drying rate. 15 plants were selected randomly from each plot for assessment of plant growth. The plant height, branch number, stem diameter, root branch number, root length, and shoot and root dry mass were measured after harvesting the *S. miltiorrhiza* plants.

2.6. Plant Nutrient Analysis

From each plot, 15 plants were selected randomly and mixed for analysis of the nutrient contents. Briefly, 100 mg of the dried plant sample was transferred to a digestion tube, in which 5 mL of a perchloric acid/sulfuric acid/water (10:1:2) solution was added. The mixture was digested using the Mars 6 microwave reaction system (CEM Corporation, Matthews, NC, USA) until a clear liquid was obtained. The digested samples were then analyzed for contents of N using the Kjeldahl method, K using K flame photometry, and P using colorimetry [35]. The levels of Ca, Mg, Zn, Cu, Fe, and Mn absorbed by the plants were analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES, Spectro CIROS, SPECTRO Analytical Instruments, Kleve, Germany) [36].

2.7. Quantitative Analysis of Phenolic Acids

Fifteen plants per plot were selected randomly and mixed. Stock solutions of rosmarinic acid and salvianolic acid B (purchased from China National Institutes for Food and Drug Control, Beijing, China) were dissolved in 70% aqueous methanol and diluted to an appropriate concentration range to establish the calibration curves. The dried roots of the plant materials were ground to a powder as described above. Individual samples (40 mesh, 100 mg) were accurately weighed, mixed with 20 mL 70% aqueous methanol and sonicated for 30 min at room temperature. The extract solution was filtered through a 0.45 μm filter, and 5 μL was injected for HPLC (Waters Corp., Milford, MA, USA) analysis. HPLC separation was performed with a mobile phase containing a gradient of solvents A and B, where A was a deionized water-phosphoric acid (A; 100:0.020, v/v) solution and B was acetonitrile. The initial condition was A-B (95:5, v/v), which changed linearly to A-B (88:12, v/v) in 12 min. The percentage of mobile phase B increased abruptly by 20% at 12.1 min, followed by linear increments to 25% over the next 20 min. The detection wavelength was set at 280 nm for analyses, and the column temperature was set at 25 °C; the flow rate was 1.0 mL/min.

2.8. Statistical Analysis

All the data were analyzed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) and tested by one-way ANOVA (analysis of variance). The least significant difference test at $p < 0.05$ was used to discriminate between the means (See Supplementary Materials Table S1).

3. Results

3.1. AMF Root Colonization

The root colonization of *S. miltiorrhiza* by *G. mosseae* reached approximately 96 ± 3% (n = 10) prior to transplantation in the field. After harvesting, the colonization rate ranged from 70% to 86% in the inoculated plants, as compared with the rate of 46% in the control plants. Most of the fungal structures were present, including hyphae, arbuscules, and vesicles (data not shown).

3.2. Effects of AMF on Field Diseases and S. miltiorrhiza Growth

Non-inoculated plants of *S. miltiorrhiza* growing in continuously cropped field suffered serious Fusarium wilt. The healthy plants and plants infected with Fusarium wilt differ in such characteristics as the aboveground biomass, leaf etiolation and browning, stem wilting, small rootlets, root rot, caulis vascular growth, and taproot browning (Figure 2). *G. mosseae* inoculation significantly decreased the disease incidence of *S. miltiorrhiza*. The dried seedling rate in the control plants was 52.6%. The inoculation with AMF significantly decreased the dried seedling rate by 75% ($p < 0.01$) (Figure 3).

G. mosseae treatment increased the shoot and root dry weight (by 48.1% and 39.2%), crown width (by 26.0%), plant height (by 25.6%), stem diameter (by 18.2%), and root length (by 19.7%), although significant differences were not observed in the leaf number, root diameter, and radical number between the plants with AMF treatment and the control plants (Table 1).

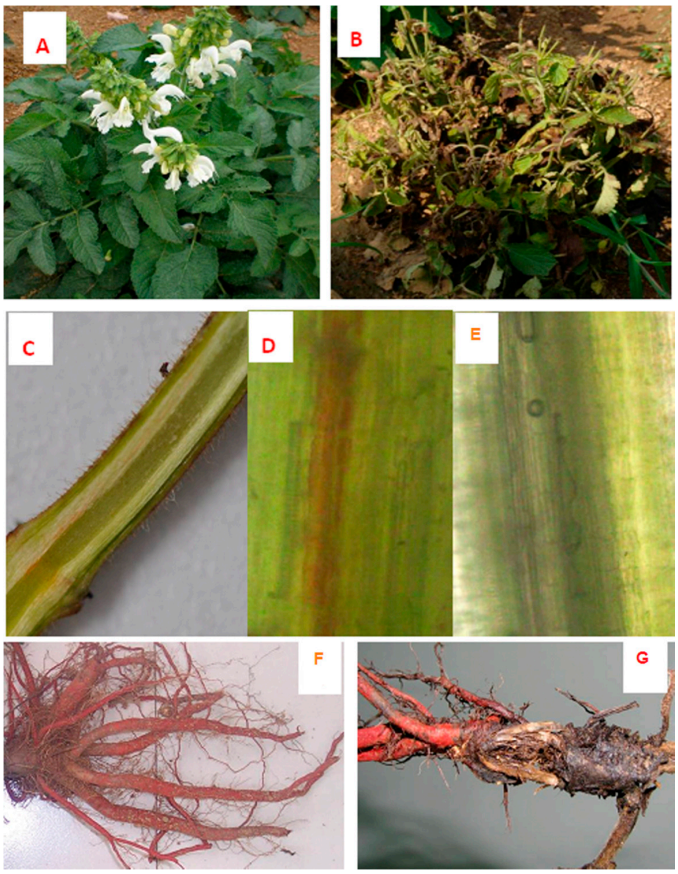

Figure 2. Symptoms of wilt disease on *S. miltiorrhiza* in the field. (**A**) Healthy plant; (**B**) Diseased plant; (**C**) Stem longitudinal section of diseased plant; (**D**) Stem longitudinal section of diseased plant (×100); (**E**) Stem longitudinal section of healthy plant (×100); (**F**) Root of a healthy plant; (**G**) Root of a diseased plant.

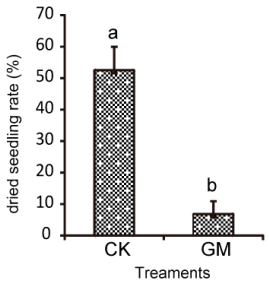

Figure 3. Effect of *G. mosseae* on dried seedling rate of *S. miltiorrhiza*. CK: non-inoculated plants; GM: plants inoculated with *G. mosseae*; Within each column, values associated with a different lowercase letter (a and b) differ from one another significantly ($p \leq 0.05$).

Table 1. Effect of *G. mosseae* on *S. miltiorrhiza* growth parameters.

Treatments	Biomass of Shoot (g)	Biomass of Root (g)	Leaf Number	Crown Width (cm)	Plant Height (cm)	Stem Diameter (cm)	Radical Number	Root		
								Length (cm)	Diameter (cm)	Number
CK	36.44 ± 1.70 ^{b}	34.11 ± 3.19 ^{b}	74.74 ± 11.99 ^{a}	38.56 ± 1.62 ^{b}	39.58 ± 2.67 ^{b}	1.97 ± 0.09 ^{b}	4.31 ± 1.89 ^{a}	30.60 ± 0.79 ^{b}	1.82 ± 0.16 ^{a}	31.48 ± 2.47 ^{a}
GM	53.97 ± 3.87 ^{a}	47.47 ± 2.87 ^{a}	84.75 ± 4.14 ^{a}	42.66 ± 1.04 ^{a}	49.86 ± 1.72 ^{a}	2.33 ± 0.08 ^{a}	7.70 ± .93 ^{a}	36.64 ± 2.23 ^{a}	1.99 ± 0.19 ^{a}	30.82 ± 0.43 ^{a}

CK: non-inoculated plants; GM: plants inoculated with *G. mosseae*. Values shown are means ± SE (standard error) (n = 3). Within each column, values associated with a different lowercase letter (a and b) differ from one another significantly ($p \leq 0.05$).

3.3. Effect of *G. mosseae* on Phenolic Acids in *S. miltiorrhiza*

Water-soluble phenolic acids such as salvianolic acid B and rosmarinic acid are among the major bioactive constituents within *S. miltiorrhiza*. Inoculation with *G. mosseae* significantly promoted salvianolic acid B synthesis and increased salvianolic acid B concentrations in the shoots (by 21.9%) and roots (by 9.2%) of *S. miltiorrhiza*. Inoculation with *G. mosseae* also promoted the synthesis of rosmarinic acid in *S. miltiorrhiza*, although the changes in their contents were not statistically significant (Table 2, Figure 4).

Table 2. Effect of *G. mosseae* on water-soluble constituents' content in *S. miltiorrhiza*.

Treatments		Rosmarinic Acid (%)	Salvianolic Acid B (%)
Shoot	CK	0.89 ± 0.02^{a}	1.14 ± 0.02^{b}
	GM	0.63 ± 0.09^{b}	1.39 ± 0.09^{a}
Root	CK	0.33 ± 0.03^{a}	0.76 ± 0.00^{b}
	GM	0.34 ± 0.02^{a}	0.83 ± 0.01^{a}

GM: plants inoculated with *G. mosseae*; CK: non-inoculated plants. Values shown are means ± SE (standard error) (n = 3). Within each column, values associated with a different lowercase letter (a and b) differ from one another significantly ($p \leq 0.05$).

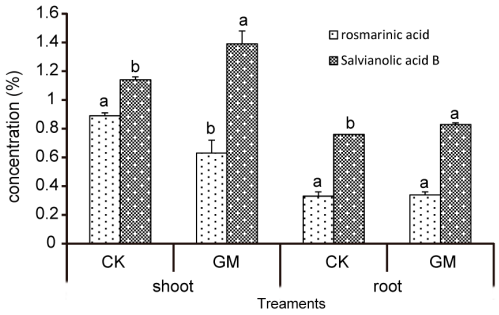

Figure 4. Effect of *G. mosseae* on water-soluble constituents in *S. miltiorrhiza.* CK, control plants without *G. mosseae* inoculation; GM, plants with *G. mosseae* inoculation. Within each column, values associated with a different lowercase letter (a and b) differ from one another significantly ($p \leq 0.05$). The error bar represents the standard error (n = 3).

3.4. Effect of AMF on Plant Mineral Nutrient Status

The plant mineral nutrients include macroelements and microelements. In terms of the macroelements, inoculation with *G. mosseae* significantly increased K concentration in *S. miltiorrhiza* shoot by 24.3%. The inoculated *S. miltiorrhiza* showed reduced P concentrations in the shoots and roots and Mg concentration in the roots. Significant differences were not observed in the concentrations of N and Ca in the shoots and roots, and Mg in the shoots between the AMF-inoculated plants and the control plants (Table 3).

Table 3. Effect of *G. mosseae* inoculation on element concentration in shoots and roots of *S. miltiorrhiza*.

Treatments		N (g/kg)	P (g/kg)	K (g/kg)	Ca (g/kg)	Mg (g/kg)	Cu (mg/kg)	Zn (mg/kg)	Fe (g/kg)	Mn (mg/kg)
Shoot	CK	16.40 ± 0.75^{a}	4.31 ± 0.28^{a}	26.08 ± 0.91^{b}	2.37 ± 0.05^{a}	5.03 ± 0.12^{a}	16.41 ± 1.11^{a}	75.03 ± 6.68^{a}	1.48 ± 0.12^{a}	65.93 ± 1.07^{c}
	GM	18.26 ± 1.07^{a}	3.24 ± 0.11^{b}	32.41 ± 0.24^{a}	2.48 ± 0.06^{a}	4.29 ± 0.24^{a}	17.87 ± 0.38^{a}	74.28 ± 3.04^{a}	1.20 ± 0.12^{b}	127.49 ± 10.2^{a}
Root	CK	15.21 ± 0.94^{a}	2.87 ± 0.03^{a}	10.35 ± 0.11^{a}	0.53 ± 0.03^{a}	2.67 ± 0.02^{a}	18.01 ± 0.29^{a}	44.01 ± 2.49^{a}	0.76 ± 0.03^{b}	33.8 ± 1.33^{b}
	GM	13.88 ± 0.67^{a}	2.51 ± 0.04^{b}	10.07 ± 0.26^{a}	0.54 ± 0.01^{a}	2.45 ± 0.07^{b}	21.03 ± 1.80^{a}	42.97 ± 2.40^{a}	1.33 ± 0.11^{a}	55.82 ± 5.94^{a}

CK: non-inoculated plants; GM: plants inoculated with *G. mosseae*;. Values shown are means ± SE (n = 3). Within each column, values associated with a different lowercase letter (a and b) differ from one another significantly ($p \leq 0.05$).

Inoculation with *G. mosseae* produced a more pronounced effect on the plant microelements than on the macroelements. Inoculation with *G. mosseae* significantly increased Mn concentration in the

shoots (by 93.4%) and roots (by 65.1%) and Fe concentration in the roots (by 75%) of *S. miltiorrhiza*. The inoculated *S. miltiorrhiza* showed a reduced Fe concentration in the shoots. Significant differences were not observed in the concentration of Zn in the shoots or roots between the AMF-inoculated plants and the control plants (Table 3).

4. Discussion

Arbuscular mycorrhiza is the most widespread type of mycorrhizal symbiosis, a mutualistic association between plants and fungi. AMF are found ubiquitously in soils wherever their plant hosts are available. In one of our previous surveys carried out during 2009 to 2011, we found AMF in rhizosphere soil of *S. miltiorrhiza* belonged to *Glomus*, *Gigaspora*, *Scutellospora*, and *Entrophospora*, and *Glomus* was dominant. This is compatible in a certain degree to Helgason's study on host specificity of AMF, which indicated *Glomus hoi* consistently occupied a large proportion of root systems and out-performed the other fungi [37]. Along with continuous cropping, the colonization rate of AMF in rhizosphere soil of *S. miltiorrhiza* dropped markedly. In the soil continuously cropped with *S. miltiorrhiza* for one to two years, the colonization rate of AMF was more than 80%, but in that cropped for three years, the colonization rate was only 40% (unpublished data). In this study, AMF infection rate of control plants was 46%.

On the other hand, in the 2009–2011 survey we also found that an increase in the number of years of continuous cropping was associated with an increased incidence of Fusarium wilt, which could reach 60–70% after continuous cropping for three years, a rate significantly higher than that following continuously cropping for one or two years (10–20%). The causative agent of Fusarium wilt in *S. miltiorrhiza* is *Fusarium oxysporum* (*F. oxysporum*) [16]. Fusarium wilt decreased the plant yield and reduced the effective components content [15].

Glomus mosseae inoculation can improve resistance against root and other pathogens. In tomato pre-inoculated with *G. mosseae* increased tolerance against either *Phytophthora nicotiana* var. *parasitica* [38] or *Rhizoctonia solani* [39] was observed. In the present study, we evaluated the effects of AMF *G. mosseae* on continuous cropping of *S. miltiorrhiza*. Several studies suggested that an early establishment of AMF symbiosis with the host plants reduced the time for the host plants to receive benefits from this mutualistic relationship to result in improved growth and reduced diseases [40,41], and plants pre-inoculated with mycorrhiza performed better in the field than those inoculated simultaneously with a pathogen and AMF [40]. We thus decided to sow the seeds in AMF affected soils first, and then transplant healthy seedlings into the field when the AMF root colonization rate reached over 80%. We found in 50-day-old *S. miltiorrhiza* seedlings, the root colonization by *G. mosseae* reached approximately 96%; the seedlings were suitable for field transplantation.

Our results showed inoculation with *G. mosseae* can improve the growth of continuously cultivated *S. miltiorrhiza*. The inoculation significantly decreased dry seedlings caused by Fusarium wilt; the dried seedling rate in the control plants was 52.6%, while that in the inoculated plants was only a little more than 10%. It was reported by Hage-Ahmed et al. that *G. mosseae* could control tomato soil borne diseases caused by *Fusarium oxysporum* f.sp. *lycopersici*; root exudates played an important role in plant-microbe interactions in the rhizosphere, and citrate and chlorogenic acid could be identified as possible candidates for the reduction in *Fusarium* spore germination [30]. Moreover, AMF also can inhibit the secretion of mycotoxin such as trichothecenes by *Fusarium* species, which cause serious disease on infected plants. In *Fusarium sambucinum* infected potato plants, *Glomus irregulare* inhibited the growth of *F. sambucinum* and significantly reduced the production of the trichothecene 4,15-diacetoxyscirpenol in roots and tubers, thus alleviating black dry rot of the potato tuber [42].

G. mosseae inoculation also improved biomass yield of continuously cropped *S. miltiorrhiza*. The inoculated plants showed notable increase in shoot and root dry weight, crown width, stem diameter, plant height, and root length ($p < 0.05$). This is in accord with many reports [43,44]. Arbuscular mycorrhizal fungi are well known to improve plant growth, while seedlings usually benefit more from the symbiosis than do adult plants [45]. The study also partly supported this point of view.

Continuous cropping results in an imbalance of nutrient availability, and affects the growth of plant. Zhong et al. reported banana yield decline was accompanied with continuous cropping deteriorated soil quality, which was evidenced by an increase of soil acidity; a decrease of total organic C; accumulation of N, P, K, Ca and Cu; and deficiency of Mg, S, Fe, Mn, and Zn [46]. The main effect of AM fungi in improving plant growth is through improved uptake of nutrients, both macroelements and minor elements, which is due to exploration by the external hyphae of the soil beyond the root hair [47]. Our results also showed inoculation with *G. mosseae* significantly increased K concentration by 24.3%, Mn concentration in the shoots (by 93.4%) and roots (by 65.1%), and Fe concentration in the roots (by 75%) of *S. miltiorrhiza*. Therefore, we speculated that inoculation with *G. mosseae* improved growth by improved nutrient uptake of the plant.

AMF colonization promotes the synthesis of the secondary compounds (phenolics, cyclohexenone derivatives, and terpenes) in the roots and/or shoots of many economically important crops [48–50]. We found that inoculation with *G. mosseae* could enhance the accumulation of salvianolic acid B in *S. miltiorrhiza*. Salvianolic acid B belongs to phenolic compounds, which occur naturally in plant system and, owing to their antimicrobial properties, inhibit fungal spore germination and toxin production by pathogens [51]. On pea (*Pisum sativum*) plants, *G. mosseae* colonization and total phenolic accumulation were closely correlated with powdery mildew disease (*Erysiphe pisi* infection) intensity [52]. The resistance of the mycorrhizal plants is enhanced through increased of phenolic acid synthesis in the roots [53]. Thus, we deduced that the accumulation of salvianolic acid B in mycorrhizal plants may also contribute to the tolerance of *S. miltiorrhiza* to continuous cropping.

Our results also showed that AMF inoculation significantly increased the Mn concentration in the plant roots and shoots (Table 3), during continuous cropping of *S. miltiorrhiza*. According to Sun et al., an optimal amount of exogenous Mn promoted the growth of the shoots and roots [54], whereas Mn deficiency increased the susceptibility of wheat plants to infections compared with plants adequately supplied with all essential nutrients [55]. The application of Mn was shown to decrease the intensities of a spectrum of diseases in different crops, including root rot, take-all, powdery mildew, and leaf and stem rust in cereals, damping-off and wilt in cotton, scab and late blight in potato, leaf spots in soybean, and black leaf mold on tomato, to name a few; excessive Mn above the optimum level for plant growth could contribute to the control of *Pseudocercospora fuligena* in tomato plants [56]. We therefore assume that AMF-mediated biocontrol of soil-borne diseases in *S. miltiorrhiza* is associated with increased Mn concentrations in the roots and shoots, which, in mycorrhizal plants, may serve as a mechanism for resisting the harmful effects of continuous cropping.

We found that inoculation with *G. mosseae* reduced Fe concentration in the shoots, which might also be attributed to an increased biomass of the host plant. On the other hand, the inoculation significantly increased Fe concentration in the roots of *S. miltiorrhiza* by 75%, a finding consistent with the results of a previous study. In that study, Wang et al. reported that *Glomus versiforme* had a significant effect on the contents of Fe in *Poncirus trifoliate*; the AMF-treated seedlings presented significantly higher contents of Fe compared with the control seedlings [57]. Fe is an essential micronutrient for plants and their associated microorganisms. Fe content in these organisms relies on the soil Fe supply; but in cultivated soils, the bioavailability of Fe becomes low to result in an intense competition for Fe among rhizosphere microorganisms; such competition favors the microorganisms with the most efficient Fe uptake. Competition for Fe may also occur between plants and microbes during pathogenesis [58]. Most of the plant growth-promoting rhizobacteria, such as pseudomonas, release small ligands known as siderophores, which bind available ferric ions in the rhizosphere to make them unavailable to phytopathogens and thus protect the plant's health [59,60]. Based on these observations, we believe that AMF-mediated biocontrol of soil-borne diseases that affect continuously cropped *S. miltiorrhiza* are associated with increased concentrations of Fe in the roots, which leads to a decrease in available Fe for the pathogens.

5. Conclusions

Inoculation with *G. mosseae* can alleviate continuous cropping obstacle to *S. miltiorrhiza*, as evidenced by increasing the plant's biomass and decreasing the rate of dried seedlings caused by Fusarium wilt. It can also improve salvianolic acid B accumulation in the roots, which is valuable in providing better quality crude drug. *G. mosseae* inoculation may serve as a potential approach of biocontrol to improve the performance of continuously cropped *S. miltiorrhiza*.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/7/7/692/s1, Table S1: Values of P, F, n in statistics (GM vs. CK).

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