Effect of AM fungi on the growth, photosynthetic characteristics and quality of *Salvia miltiorrhiza* Bge under different moisture and phosphorus levels

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Abstract

Effects of AM fungi on growth , photosynthetic characteristics and quality of *Salvia miltiorrhiza* under different soil moisture and phosphorus application rates was studied using pot experiment. The results showed that inoculation of AM fungi significantly increased the chlorophyll, net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate(Tr) and tanshinone II_A content of *Salvia miltiorrhiza* under the same soil moisture and phosphorus level. Under the same soil moisture and different phosphorus levels, the chlorophyll, Pn, Gs, Tr and tanshinone II_A content of *Salvia miltiorrhiza* increased firstly and then decreased with increasing phosphorus application rate, and they reached the maximum at P_2(0.15 g/kg). The content of chlorophyll, Pn, Gs and Tr respectively was 1.182 $mg\cdot g^{-1}$, 12.849 $\mu mol\cdot m^{-2}\cdot s^{-1}$, 0.286 $mmol\cdot m^{-2}\cdot s^{-1}$ and 4.428 $mmol\cdot m^{-2}\cdot s^{-1}$. Under different soil moisture and the same phosphorus levels, tanshinone II_A of inoculated plants were significantly higher than non inoculated plants. The content of medicinal ingredient was higher at 70% WHC. When at 70% WHC and phosphorus application rate was 0.15 g/kg, the content of medicinal ingredient in inoculated plants was highest, that was 0.252%.

Key words: AM fungi; Salvia miltiorrhiza Bge; Phosphorus; photosynthetic characteristics

1. Introduction

Salvia miltiorrhiza Bge, called Danshen in Chinese, was a well-known and important medicinal plant because its dried root and rhizome could remove blood stasis, induce menstruation and relieve menalgia, pure heart and soothe the nerves and so on. Tanshinone and salvianolic acid are main active ingredients of *Salvia miltiorrhiza*[1]. Tanshinone has anti-tumor, anti-oxidation and other cardiovascular pharmacological effects[2]. In recent years, with the changing human disease spectrum, as a kind of Chinese traditional medicine for prevention and treatment of several dangerous diseases, the market of *Salvia miltiorrhiza* was in great demand. Wild *Salvia miltiorrhiza* resources can't meet market demand so that planting *Salvia miltiorrhiza* become the focus of attention. However, due to using of fertilizers improperly, pesticide residues and unstandardized planting during the cultivation process[3], which resulted in its production and medicinal ingredients decreased and seriously affected the healthy development of chinese medicine industry. Thus, it was important to make use of the modern biological technology to improve the growing conditions so as to improve the yield and quality of *Salvia miltiorrhiza.*

Arbuscular mycorrhizal (AM) fungi was one of the most widely distributed in nature mycorrhizal fungi, and it could form arbuscular mycorrhizal symbiosis with about 90% of terrestrial plants on Earth. Not only it could promote absorption of mineral elements and water to plants, especially phosphate mineral elements in poor mobile, but also improve crop drought resistance, disease resistance, resilience, contribute to the growth and development of plants, increase crop yield and quality[4], and improve the accumulation of plant active ingredients, etc[5]. But the soil nutrients have a direct impact on the effect of AM fungi[6]. He chao[7]researched that inoculation AM fungi can improve photosynthetic characteristics of scuteliaria baiealensis and baicalin content in the phosphorus content of 0.15 g/kg. Tan Weidong[8]researched that inoculation AM fungi can improve artemisia annua growth and promote the accumulation of artemisinin component under the phosphorus content of 40~80 mg/kg. HE Xueli[9]researched that inoculated with AM fungi can promote Salvia root to water and mineral element absorption and utilization under different water conditions and P-applied levels. Han Jianping[10]researched that application of nitrogen equal to phosphorus was beneficial to accumulation of danshensu and tanshinone II_A. But the effects of AM fungi on the the growth, photosynthetic characteristics and medicinal components of *Salvia miltiorrhiza* were relatively few in the condition of different water and phosphorus content. This experiment studied AM fungi on the effects of growth index, photosynthetic characteristics and medicinal ingredients under the different water and phosphorus, in order to better develop the production and quality and provide a theoretical basis for improving the yield and quality of artificial planted *Salvia miltiorrhiza*.

2. Meterials and methods

2.1 Experiment materials

Salvia miltiorrhiza seeds purchased from the Anguo medicine market. Microbial inoculants obtained from rhizosphere soil containing spores and mycelium of clover inoculated with AM fungi, and each 10g dried soil contained 60 spores.

The tested soil was farmland cinnamon soil in Hebei Baoding. pH=8.18, organic matter: 13.94 $g\cdot kg^{-1}$,:alkaline hydrolysis N: 62.47 $mg\cdot kg^{-1}$, available phosphorus: 18.56 $mg\cdot kg^{-1}$, available potassium: 105.285 $mg\cdot kg^{-1}$. Over 2 mm sieve mass ratio of soil: sand = 2: 1 mixed stand-by application, increasing soil permeability and drainage properties. Pot container was hard plastic basin with a hole in bottom, specification: 21.5 cm×16 cm×20.5cm. The use of nitrogen fertilize, phosphorus fertilize and potassium fertilizer respectively was $CO(NH2)_2$, NaH_2PO_4 and K_2SO_4 and they were analytical grade. The maximum water holding capacity was 21%.

2.2 Experimental design

The experiment set soil moisture, AM fungi and phosphorus concentration three factors, while soil moisture amount set two levels, water holding capacity (WHC) of 35% and WHC of 70%. Phosphorus concentration were 0.07(P_1), 0.15(P_2), 0.23g/kg(P_3),which were set on the same moisture level. The two treatments of AM fungi inoculation and non inoculation were set on the same moisture and phosphorus concentration level, and each treatment was replicated 4 times, that was total 48 basins.

Seeding on 2014-04-10. Three kilogram soil for each pot, each pot adds 0.963g $CO(NH_2)_2$ and 1.005g K_2SO_4. Each inoculation treatment adds inoculants 40g, while the control sample adds equal amount of sterilizing inoculants, made sure each pot 3 trees after emergence. Two months later, start water treatment, control moisture by gravimetric method on 2014-07-5. During the test, the reasonable control of the moisture and temperature, regular watering, pest control, weeding, harvested on 2014-9-5.

2.3 Experimental methods

Soil pH was measured by pH meter. Alkaline hydrolysis N was measured by alkaline hydrolysis diffusion method. Available P was measured by Mo-Sb colorimetry method. Organic matter was determined by Potassium dichromate volumetric method. Available K was measured by nephelometry[11]. Dry mass of overground and underground were measured by scale after harvested. The chlorophyll was extracted by acetone overnight, and then determined by UV spectrophotometer[12]. Mycorrhizal infection rate was measured by Biermann and Linderman method[13]. Net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate(Tr) in the leaf of *Salvia miltiorrhiza* were measured by Li-6400 portable photosynthesis meter. Tanshinone II_A was extracted by toluene and determined by HPLC[14].

3. Results and analysis

3.1 Effects of AM fungi on the growth of *Salvia miltiorrhiza* under different moisture and P levels.

It can be seen from Table 1, on the same soil moisture and phosphorus level, Mycorrhizal infection rate of inoculated plants was significantly higher than non inoculated plant. In contrast, On 70% WHC and P3 level, Mycorrhizal infection rate of non inoculated plants was higher than inoculated plant. But the difference was not significant (P>0.05). On the same soil moisture and different phosphorus levels, mycorrhizal infection rate between inoculated and non-inoculated plants was significantly different. The order was P_2> P_3> P_1. On the same phosphorus level and different soil moisture, mycorrhizal infection rate was higher at 70% WHC.

on the same soil moisture and phosphorus level, Compared with non inoculated plants, inoculating AM fungi significantly increased the dry mass overground and underground parts at P_1~P_2 level. Under 35% WHC and P_3 level, dry mass overground and underground part of inoculated plants were significantly higher than non inoculated plant. In contrast, on 70% WHC and P_3 level, dry mass overground and underground part of non inoculated plants were higher than inoculated plant. But the difference was not significant (P>0.05).

On the same soil moisture and different phosphorus levels, the dry mass overground and underground parts of *Salvia miltiorrhiza* increased firstly and then decreased with increasing phosphorus application rate, and they reached the maximum at P_2(0.15 g/kg). The dry mass overground at 35% WHC for inoculated plants and the dry mass overground at 70% WHC for inoculated plants, whose difference between P_1 and P_3 level were not significant. the difference between other phosphorus levels were significant (P <0.05). On the same phosphorus level and different soil moisture, dry mass overground and underground parts were higher at 70% WHC.

Table 1 Effects of AM fungi on the growth of *Salvia miltiorrhiza* under different moisture and P levels

Water content(%)	Inoculation	P treatments	Infection rate(%)	Dry mass(g)	
				Overground	Underground
35%	AM	P1	63.24±0.98C*	1.798±0.044B*	2.055±0.060C*
		P2	84.67±0.94A*	2.168±0.053A*	2.979±0.017A*
		P3	74.25±1.09B*	1.863±0.017B*	2.326±0.031B*
	CK	P1	55.68±1.53c	1.451±0.041c	1.525±0.084c
		P2	69.84±1.46a	2.052±0.047a	2.596±0.075a
		P3	63.72±0.66b	1.563±0.012b	1.742±0.085b
70%	AM	P1	77.24±0.86F*	2.049±0.072F*	2.799±0.128E*
		P2	94.56±0.86D*	2.894±0.026D*	4.576±0.035D*
		P3	79.58±0.70E	2.296±0.099E	2.826±0.059E
	CK	P1	69.2±0.63f	1.765±0.021f	2.134±0.087f
		P2	82.5±1.02d	2.349±0.003d	3.295±0.016d
		P3	78.29±0.72e	2.185±0.011e	2.896±0.061e

Note: The date is “mean ± standard deviation”. The sign * means significant difference between inoculated and non-inoculated plants under same moisture and P levels. Under different P levels, different capital letters mean significant difference among inoculated plants under the same moisture levels. Under different P levels, different lowercases mean significant difference among non-inoculated plants under the same moisture levels. All the significant differences are at 5% level. The same below.

3.2 Effects of AM fungi on the photosynthesis of *Salvia miltiorrhiza* under different moisture and P levels

It can be seen from Table 2, on the same soil moisture and phosphorus level, Compared with non inoculated plants, inoculation of AM fungi significantly increased the chlorophyll, net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate(Tr) at P_1 ~P_2 level. The chlorophyll and transpiration rate(Tr) of inoculated plants were significantly higher than non inoculated plant at P_3 level. On 35% WHC and P_3 level, net photosynthetic rate (Pn) of inoculated plants were significantly higher than non inoculated plant($P<0.05$). However, on 70% WHC and P_3 level, net photosynthetic rate (Pn) of inoculated plants were higher than non inoculated plant. But the difference was not significant ($P>0.05$). On 35% WHC and P_3 level, stomatal conductance (Gs) of inoculated plants were higher than non inoculated plant. But the difference was not significant ($P>0.05$). In contrast, on 70% WHC and P_3 level, stomatal conductance (Gs) of non inoculated plant were significantly higher than inoculated plant.

On the same soil moisture and different phosphorus levels, the chlorophyll, net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate(Tr) of *Salvia miltiorrhiza* increased firstly and then decreased with increasing phosphorus application rate, and they reached the maximum at P_2(0.15 g/kg). In addition, they were significantly higher than P_1 and P_3 level. The difference between each phosphorus levels was significant ($P<0.05$), which show that phosphorus level has a great influence on the photosynthesis of *Salvia miltiorrhiza*.On the same phosphorus level and different soil moistur, the chlorophyll, net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate(Tr) were higher at 70% WHC, which show that water stress has a great influence on the photosynthesis of *Salvia miltiorrhiza*.

Table 2 Effects of AM fungi on the photosynthesis of *Salvia miltiorrhiza* under different moisture and P levels

Water content(%)	Inoculation	P treatments	Chlorophyll (mg.g^{-1})	Pn/(μmol·m^{-2}·s^{-1})	Gs/(mmol·m^{-2}·s^{-1})	Tr/(mmol·m^{-2}·s^{-1})
35%	AM	P1	1.405±0.010B*	10.936±0.048B*	0.164±0.009B*	3.324±0.083B*
		P2	1.753±0.010A*	11.764±0.085A*	0.196±0.010A*	3.852±0.08A*
		P3	1.359±0.012C*	10.635±0.047C*	0.134±0.010C	3.058±0.057C*
	CK	P1	1.123±0.010c	10.022±0.066c	0.144±0.008b	2.394±0.12c
		P2	1.304±0.017a	10.998±0.081a	0.167±0.007a	3.368±0.078a
		P3	1.169±0.010b	10.247±0.081b	0.123±0.01c	2.729±0.077c
70%	AM	P1	1.624±0.014E*	11.672±0.090E*	0.189±0.01E*	3.726±0.112E*
		P2	1.812±0.019D*	12.849±0.098D*	0.286±0.023D*	4.428±0.062D*
		P3	1.554±0.012F*	11.383±0.090F	0.142±0.013F	3.369±0.055F*
	CK	P1	1.302±0.008e	11.125±0.074f	0.143±0.015f	3.446±0.079e
		P2	1.498±0.012d	11.958±0.093d	0.234±0.013d	3.926±0.053d
		P3	1.225±0.015f	11.287±0.049e	0.174±0.009e*	3.297±0.017f

3.3 Effects of AM fungi on the the content of tanshinone II$_A$ of *Salvia miltiorrhiza* under different moisture and P levels

It can be seen from Fig.1, on the same soil moisture and phosphorus level. The content of tanshinone II$_A$ in inoculated plants were higher than non inoculated plants. However, on 35% WHC and P_3 level, tanshinone II$_A$ in non inoculated plants were higher than inoculated plants. On the same soil moisture and different phosphorus levels, the tanshinone II_A content of *Salvia miltiorrhiza* increased firstly and then decreased with increasing phosphorus application rate, and they reached the maximum at P_2(0.15 g/kg). In addition, they were significantly higher than P_1 and P_3 level. It showed that On the same phosphorus level and different soil moistur, the content of medicinal ingredients of *Salvia miltiorrhiza* were higher at 70% WHC than that at 35% WHC, which shows that phosphorus can promote the accumulation of medicinal ingredients. But the too high or too low concentration of phosphorus will affect the accumulation of medicinal ingredients. The HPLC of tanshinone II_A extraction for inoculated plants on the level of 70% WHC and P_2 was analyzed. The mass fraction of tanshinone II_A was 0.252% after calculated that was in line with the provisions of Chinese Pharmacopoeia. Tanshinone II_A was more than 0.2%.

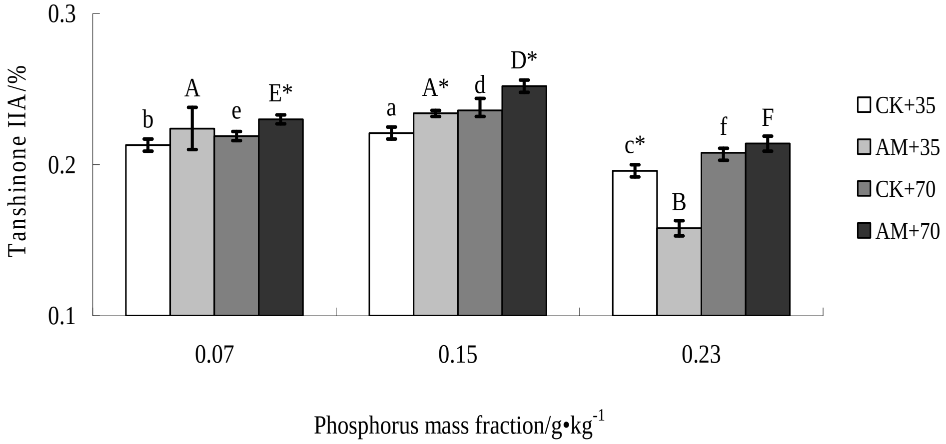

Fig.1 The content of tanshinone II_A in *Salvia miltiorrhiza* with AM fungi under different moisture and P levels

Note: The sign * means significant difference between inoculated and non-inoculated plants under same moisture and P levels. Under different P levels, different capital letters mean significant difference among inoculated plants under the same moisture levels. Under different P levels, different lowercases mean significant difference among non-inoculated plants under the same moisture levels. All the significant differences are at 5% level.

4. Discussion

The results further confirmed that AM fungi could form good symbiotic relationship with *Salvia miltiorrhiza*. AM fungi form huge mycelium network around the host plant roots , expand roots on soil moisture and nutrients in the contact area and increased root absorption beyond rhizosphere soil moisture and phosphorus, thereby it could improve the moisture and nutrient status in plants[15]. In addition, AM fungi form symbiotic with the host, which is conducive to promote the development of its root[16]. Thus, AM fungi could significantly increased the dry mass overground and underground parts of *Salvia miltiorrhiza*, which was almost consistent with the inoculation effect of *Camptotheca acuminate*[17]. In this experiment, compared with non inoculated plants, inoculation with AM fungi significantly increased the mass fraction of chlorophyll in fresh leaves of *Salvia miltiorrhiza*, which was almost consistent with the inoculation effect of *Bupleurum*[18]. The reason may be that inoculation AM fungi promote absorption of phosphorus in *Salvia miltiorrhiza.* Phosphorus is an integral component of many coenzyme. These coenzyme involved in respiration and photosynthesis of plants and promote the synthesis, conversion and transmission of carbohydrate[19], which promote chlorophyll synthesis, enhance photosynthesis of *Salvia miltiorrhiza*. In this experiment, net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate(Tr) in *Salvia miltiorrhiza* at 70% WHC were higher than that at 35% WHC .The reason may be that water stress could cause leaf stomatal closure, cell loss moisture[20-21], destruction of moisture metabolism in plants[22], decreasing activity of enzyme in Calvin cycle and the degradation of Rubisco[23], which result in damaging photosynthetic organ and Inhibiting photosynthesis of Salvia miltiorrhiza[24-25].

In this experiment, inoculation with AM fungi significantly increased the mass fraction of tanshinone II_A. When at 70% WHC and phosphorus application rate was 0.15 g/kg, the content of medicinal ingredient in inoculated plants was highest, that was 0.252%. At this time, the soil nitrogen: phosphorus=0.149: 0.15, which was almost consistent with Han jianping's research result that optimum nitrogen and phosphorus ratio=1: 1[9].The too high or too low concentration of phosphorus will affect thethe growth of *Salvia miltiorrhiza* and accumulation of tanshinone II_A. The reason may be that too high or too low concentration of phosphorus affect good symbiotic relationship between AM fungi and *Salvia miltiorrhiza*[6]**.** Too low concentration of phosphorus not only can't meet the growth of *Salvia miltiorrhiza*, but also can't meet the growth of AM fungi needing of phosphorus nutrition, which restrict the growth of mycorrhizal. Too high concentration of phosphorus reduce root cell membrane permeability of *Salvia miltiorrhiza* and inhibit the growth of AM fungi[26]**,** which affect absorption of phosphorus and other required elements in *Salvia miltiorrhiza*. however, these elements may affect the growth, photosynthesis and synthesis of tanshinone II_A in *Salvia miltiorrhiza.* In this experiment, the growth and the mass fraction of tanshinone II_A in *Salvia miltiorrhiza* at 70% WHC were higher than that at 35% WHC. The reason may be that water stress affect AM fungi infecting the root of *Salvia miltiorrhiza* and reduce absorption of phosphorus and other required nutrients in *Salvia miltiorrhiza*[9]. However, these nutrients may affect the growth increasing and synthesis of tanshinone II_A in *Salvia miltiorrhiza*, or soil moisture may be a direct impact on the growth and the accumulation of tanshinone II_A in *Salvia miltiorrhiza*[27]. Graham[28] researched that AM fungal hyphae may play a role as a bridge under water stress, which connected roots and water that was difficult to absorb under water stress and reduced the inhibition of water stress on mycorrhizal infection. Thus, the content of tanshinone II_A in inoculated plants were higher than non inoculated plants.

5. Conclusion

The results showed that soil moisture, phosphorus application rate and AM fungi have a positive effect on increasing growth of *Salvia miltiorrhiza*, improving photosynthesis and the accumulation of tanshinone II_A. When at 70% WHC and phosphorus application rate was 0.15 g/kg inoculation of AM fungi in *Salvia miltiorrhiza* had the highest medicinal value.

6. References

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