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ARTICLE

Mycorrhizal Fungal Effects on Growth, Antioxidant Capacity, and Medicine Quality of *Paris polyphylla* var. *yunnanensis*

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ABSTRACT

A field experiment was conducted to determine the effects of two commercial strains composed of mulple arbuscular mycorrhizal fungi (AMF) species on plant growth, antioxidant capacity, and medicine quality of *Paris polyphylla* var. *yunnanensis* in three subtropical soils from Wanzhou, Anshun and Baoshan in fields. The results showed that AMF inoculation enhanced the fungal colonization rate and activities of both succinate dehydrogenase and alkaline phosphatase, thereby, enhancing the mycorrhizal viability of *P. polyphylla* var. *yunnanensis*. The concentrations of photosynthetic pigments (chlorophyll *a*, *b*, *a* + *b* and carotenoids), soluble sugar, soluble protein and photosynthetic capacity were higher in AMF-inoculated plants than in non-AMF-treated plants in field. AMF-treated plants recorded higher activities of catalase, peroxidase and superoxide dismutase, and caused the reduction in malondialdehyde content, indicating lower oxidative damage, compared with non-AMF plants. Polyphyllin I, Polyphyllin II, Polyphyllin III, Polyphyllin IV and total polyphyllin contents were increased by AMF treatment. In conclusion, AMF improved the plant growth, antioxidant capacity and medicinal quality of *P. polyphylla* var. *yunnanensis* seedlings. Hereinto, AMF effects on the soil from Wanzhou was relatively greater than on other soils.

KEYWORDS

Paris polyphylla var. *yunnanensis*; arbuscular mycorrhizal fungi; growth and development; medicine quality

1 Introduction

Paris polyphylla var. *yunnanensis* is one of the most valuable medicinal herbs mostly distributing in Provinces of Sichuan, Guizhou and Yunnan, the southwest of China, and has been used as a traditional Chinese medicinal plant for its rhizomes containing Polyphyllin I, Polyphyllin II, Polyphyllin VI, Polyphyllin VII [1], pennogenin and diosgenin as the aglycones [2,3]. *P. polyphylla* var. *yunnanensis* is used for pain relief, detoxification, analgesic and anti-inflammatory properties [3,4]. More than 80% of traditional Chinese medicines comes from wild resources [5]. Wild individuals have been overexploited for the last few decades because of increasing demand for such medicines. And there is no effective supply available from cultivation. Many medicinal species have been listed as endangered species including *P. polyphylla* var. *yunnanensis* [4,6].

Arbuscular mycorrhizal fungi (AMF) are the most common symbiotic association between some soil fungi and plant roots [7]. AMF play a significantly stimulating role in uptake of certain nutrients (especially phosphorous and nitrogen) and water to their host plants, while the fungus obtains photosynthetically derived carbon compounds from their host plants [7]. An increasing number of experiments showed that AMF provided several benefits to their host plants, including promoted plant transplant survival rate [8], increased abiotic stress tolerance [9,10], improving soil structure and fertility [11], and disease resistance, adjusted plant population and community structure, and maintained ecosystem stability [12]. AMF inoculation is also known to have tremendous effects on plant growth by enhancing macronutrient content (P, K, and Ca) and micronutrient content (Cu, Fe, and Zn) [13], increasing biomass of medicinal plants [14], improving photosynthesis [9,15], and inducing change of alkaloids and terpenoids [16].

However, there is little information about successful inoculation of *P. polyphylla* var. *yunnanensis* with AMF or about the application of AMF inoculation to commercial *P. polyphylla* var. *yunnanensis* production. The aim of this experiment was to investigate the effect of AMF on photosynthetic pigments, membrane lipid peroxidation, antioxidant enzyme activity, and medicine quality of *P. polyphylla* var. *yunnanensis* plants, in order to further understand the application of AMF in the production of medicinal plants.

2 Materials and Methods

2.1 Mycorrhizal Fungal Inoculums

We selected two mixed AMF inoculums: 1) The AMF biofertilizer was rich in endomycorrhizal fungi *Scutellospora calospora*, *Cetraspora pellucida*, *Racocetra coralloidea* and *Racocetra fulgida* (S1); 2) The other AMF inoculum was rich in endomycorrhizal fungi *Scutellospora calospora*, *Cetraspora pellucida*, *Gigaspora margarita*, *G. gigantea*, *Septoglomus deserticola* and *Claroideoglomus claroideum* (S2). The mixed AMF treatment exhibited a superior effect on nutrient acquisition and fruit quality of plant in field than single AM fungal inoculation [17]. The two mixed mycorrhizal inoculums were obtained from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM, http://invam.wvu.edu). The inoculum consisted of extraradical hyphae, spores, and infected roots.

2.2 Plant Culture and Experimental Design

One-year-old *Paris polyphylla* var. *yunnanensis* seedlings were used, and soils were fertilized with organic sources of nutrients before transplanting seedlings. The study was located in Sichuan, Guizhou and Yunnan provinces, southwest China. The field experiment was carried out according to a completely randomized block design with three treatments (two AMF groups (S1 and S2) and a control group [CK group]) having four replicates per treatment. To examine the effect of AMF on growth of *P. polyphylla* var. *yunnanensis* seedlings, the 20 g of each inoculum containing approximately 120 spores was supplied to each plot and then mixed with the soil–sand mixture. Transplanted *P. polyphylla* var. *yunnanensis* seedlings were watered once three days to reach around 60% water contents in soils. From July 12 to 20, 2016, the *P. polyphylla* var. *yunnanensis* seedlings were inoculated with AMF at the time of transplantation. Growth characteristics, photosynthetic pigments contents and photosynthetic parameters were determined for 10 individual plants in different fields. At the same time, the leaves were harvested directly into liquid nitrogen and stored at −40°C. From November 21 to 30, 2016, the rhizomes and roots of *P. polyphylla* var. *yunnanensis* were selected for quality analysis. Meanwhile, the root was cut into 1.0–1.5 cm long root segments in an ice water bath. One part was fixed in FAA fixative and used for the determination of mycorrhizal infection rate; the other part was stored in liquid nitrogen and used for the determination of phosphatase and succinate dehydrogenase activities in mycorrhizal hyphae.

2.3 Variable Determinations

2.3.1 Mycorrhizal Colonization Rate

Assessment of roots for AMF colonization was made on those plants sampled for soil surface (0–200 mm depth). A fraction of the roots (<1 cm long) were carefully washed, and were fixed in

formalin acetic acid solution before estimation of mycorrhizal colonization [18]. The AMF colonization was estimated using a modified method of Brundrett et al. [19]. These observations were stained with 0.05% trypan blue, washed in 50% glycerol, and measured by using Olympus BX50 (Olympus, Japan) transmitted-light bright field microscope for mycorrhizal colonization. The rate of AMF colonization was determined according to the method of Trouvelot et al. [20].

2.3.2 Phosphatase and Succinate Dehydrogenase Activity

Sites of alkaline phosphatase (ALP) activity was assayed by the method of Van Aarle et al. [21]. Roots were stained for 30 min at room temperature in the dark after which they were thoroughly washed with a Tris buffer (pH 8.0). Clearing was done at room temperature for 2 h. The clearing solution contains 15 units ml1 cellulase, 0.05% sorbitol, 15 units ml1 pectinase and 0.05 M Tris/citric acid (pH 9.2). Sites of phosphatase activity were revealed by a dark purple precipitate.

Succinate dehydrogenase (SDH) activity was measured according to the method of MacDonald and Lewis [22]. 0.2 g 1 cm viable mycorrhizal fresh roots was incubated at room temperature overnight in an NBT-succinate solution. The NBT solution consist of Tris–HCl buffer (0.05 M; pH 9.2), pectinase (15 $U \cdot mL^{-1}$), cellulase (15 $U \cdot mL^{-1}$) and sorbitol (50 $g \cdot L^{-1}$). Roots were rinsed three times with distilled water, and cleared exclusively with KOH turn dark.

2.3.3 Chlorophyll (Chl) Content

Chlorophyll content was assayed based on the method of Arnon [23]. 100 mg fresh leaf tissue from the third fully expanded leaf with a mixture containing absolute ethanol until the pellets became colorless. The concentration was calculated from the value of A_{470}, A_{645} and A_{663}, and expressed as $mg \cdot g^{-1}$ FW.

2.3.4 Photosynthetic Parameters

Net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s) and transpiration rate (E) were measured using a portable photosynthesis system (Li-Cor 6400, Li-Cor Inc., Nebraska, USA). Data were recorded between 9:30 and 11:30 am during the treatment period. Plants were measured under PPFD of 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 25 ± 3°C, 80% humidity and CO_2 concentration of 500 $\mu mol \cdot s^{-1}$. Five representative plants were randomly selected from each treatment.

2.3.5 MDA Content

The content of malondialdehyde (MDA) was determined by the thiobarbituric acid (TBA) test according to the method of Liu et al. [24] and Dhindsa et al. [25]. Fresh leaf tissues (500 mg) were homogenized in 5 mL of phosphate buffer (0.05 M, pH 7.8) using mortar and pestle, and then centrifuged at 12,000 × g for 20 min. 1 mL of supernatant, 2 mL of 0.5% TBA and 1 mL of PBS buffer (pH 7.8) were incubated in boiling water for 15 min. The concentration was measured using a spectrophotometer at 532 nm and 600 nm.

2.3.6 Soluble Sugar Content

For determination of soluble sugar content, leaf samples (0.5 g) were ground in liquid nitrogen and homogenized in 10 mL of 80 % (v/v) ethanol [26]. The mixture was extracted in a water bath at 80°C for 15 min, and the supernatant was centrifuged three times (10 000 g, 20 min). Absorbance was recorded at 630 nm to measure soluble sugar content by the colorimetry of sulfuric acid-anthrone method.

2.3.7 Protein Content

The content of protein was determined at 595 nm as described by Bradford [27] using bovine serum albumin as a protein standard. Fresh leaves (0.5 g) were homogenized in distilled water. 1 mL of supernatant and 5 mL of Coomassie's Brilliant Blue solution were placed in tubes.

2.3.8 Antioxidant Enzymes Activity

A 1.0 g leaves was homogenized in precooled PBS buffer (pH 7.0), and centrifuged at 11 500 g and 4°C for 15 min. The supernatant was used for enzyme activity assay.

The catalase (CAT) activity was measured using the method of Fu et al. [28] by monitoring a change in absorbance at 240 nm for 1 min. The reaction mixture contained 25 mM sodium phosphate buffer (pH 7.0) and 0.1 mL enzyme fraction. The reaction was initiated by adding 10 mM H_2O_2.

Peroxidase activity was assayed by the method of Polle et al. [29]. The reaction mixture was composed of 50 mM potassium phosphate buffer (pH 6.5), 5 mM hydrogen peroxide (H_2O_2), 30 mL diluted enzymatic extract and 20 mM pyrogallol (benzene-1,2,3-triol), totaling 1.0 mL. The reaction was initiated by adding 0.2 mL crude enzyme preparations. The activity of POD was expressed as $\mu mol \cdot min^{-1} \cdot g^{-1}$ (FM). A change in absorbance was read at 470 nm at every 1 min for 5 min.

The method of El-Shabrawi et al. [30] was followed to measure superoxide dismutase (SOD) activity. The reaction mixture contained 0.2 mL of 13 mM methionine, 0.2 mL of 25 mM nitroblue tetrazolium (NBT), 2.4 mL of 50 mM PBS (pH 7.8), 0.1 mL of EDTA, and 50 mL of the enzyme extract. A change in absorbance was read at 560 nm.

2.3.9 Biomass

Fresh weight (FW) and dry weight (DW) of *P. polyphylla* var. *yunnanensis* were harvested in November 2016, and dried at 35°C for 48 h to obtain dry weight. Drying rate (DR) was defined as the ratio of fresh weight to dry weight.

2.3.10 Medicine Quality

The healthy rhizomes of *P. polyphylla* var. *yunnanensis* were collected in different sites and stored in sealed plastic bags at 4°C. Polyphyllin I (batch No. 111590-201103), Polyphyllin II (batch No. 111591-201103), Polyphyllin VI (batch No. 111592-201103) and Polyphyllin VII (batch No. 111593-200402) were purchased from the National Institute for Food and Drug Control (Beijing, China). Acetonitrile (HPLC-grade) was purchased from Fisher (USA). All regents were all of analytical grade and were passed through membrane filter (0.22 mm) before use to purify. Polyphyllin I, Polyphyllin II, Polyphyllin VI and Polyphyllin VII content were determined using the method previously described by Yuangui et al. [1]. Polyphyllin content was measured under column oven temperature of 40°C, flow rate of 0.25 $mL \cdot min^{-1}$, and injection volume of 5 mL. The methanol was linearly 50%, and held for 10 min before the next injection. Acetonitrile and water were used as mobile phases. The mass spectrometer (Agilent, Agilent Quick Probe, USA) was set at 350°C of gas temperature and 12 $L \cdot min^{-1}$ of gas flow.

2.4 Statistical Analysis

Statistical analysis was conducted using the *SPSS* (21.0, International Business Machines Corporation, USA) software. The experimental data of treatment and control were analyzed by using analysis of variance (*ANOVA*) with Duncan's multiple range test at 0.05 level. The figure was drawn by the OriginPro 9.1 software (*OriginLab*, Northampton, MA, USA).

3 Results

3.1 Mycorrhizal Colonization Rate in Paris polyphylla var. yunnanensis

Under natural environment, the AMF colonization rate of non-inoculated *P. polyphylla* var. *yunnanensis* plants was 51.06% (Wanzhou), 25.37% (Anshun) and 44.72% (Baoshan), respectively (Fig. 1). The AMF colonization rate was higher in AMF-inoculated than non-AMF-inoculated plants (Fig. 1). The maximum AMF colonization rate was observed inoculated plants by S1 treatment (149.09%) in Wanzhou, followed by S2 treatment inoculated plants in Wanzhou (148.18%). Exogenous AMF had a promoting effect on the mycorrhizal infection rate, indicating that it is feasible to improve the quality of *P. polyphylla* var. *yunnanensis*.

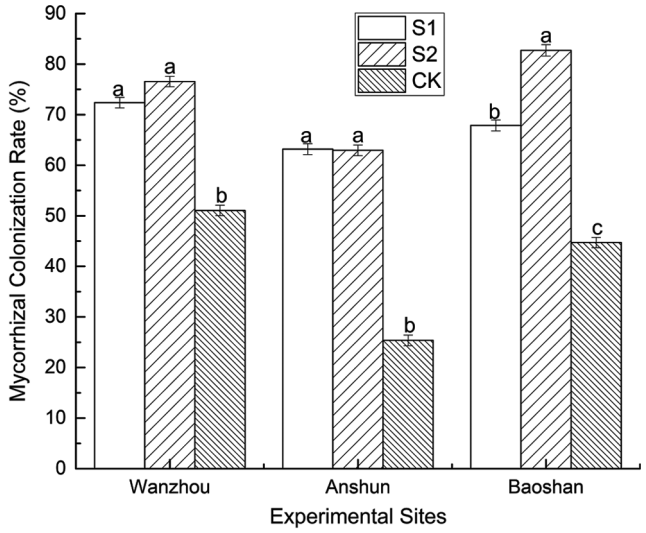

Figure 1: Effects of AMF on mycorrhizal colonization rate of seedlings in *Paris polyphylla* var. *yunnanensis*

Data (means ± SE, $n = 4$) are the difference between treatments. *Different letters* above horizontal lines indicate significant differences between treatments. The same as below.

3.2 Effects of AMF on the SDH and ALP Activities in Paris polyphylla var. yunnanensis

Succinic dehydrogenase (SDH) and alkaline phosphatase (ALP) activities were significant increased by AMF inoculations (Fig. 2). In Wanzhou, exogenous inoculation of AMF treatment caused the significant increase in the SDH and ALP by 45.52% and 60.06% under S1 conditions and by 36.39% and 60.06% under S2 conditions, respectively. In Anshun, S1 treatment caused 44.50% and 130.13% remarkable increase in the above indicators, while S2 treatment, they were 43.72% and 130.13% compared to non-AM seedlings, respectively. In Baoshan, S1 treatment caused 78.38% and 159.39% prominent ($p < 0.05$) increase in the above indicators, while S2 treatment, they were 106.90% and 159.39% compared to non-AMF seedlings, respectively.

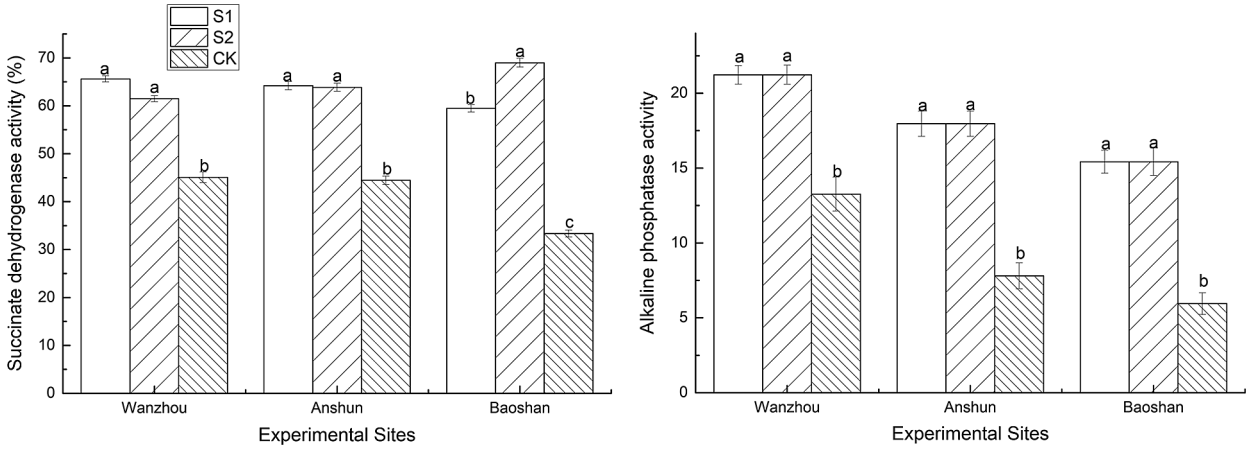

Figure 2: Effects of AMF on SDH and ALP activities of seedlings in *Paris polyphylla* var. *yunnanensis*

3.3 Effects of AMF on the Photosynthetic Pigments Content in Paris polyphylla var. yunnanensis

AMF-inoculated seedlings had higher carotenoid (Car), chlorophyll (Chl) a, Chl b and total Chl content than corresponding non-AMF-treated seedlings (Tab. 1). In Wanzhou, AMF treatments caused 27.09%, 28.59%, 15.21% and 32.45% significant ($p < 0.05$) increase in the Car, Chl a, Chl b and total Chl content under S1 conditions and 32.90%, 38.78%, 22.71% and 42.56% under S2 conditions, compared to non-AM seedlings, respectively. In Anshun, compared with the controls, mycorrhiza-inoculated plants showed 21.65%, 35.26%, 33.73% and 34.91% significantly higher Car, Chl a, Chl b and total Chl content under S1 conditions and 20.98%, 31.86%, 33.25% and 32.18% under S2 conditions, respectively. In Baoshan, compared with the control plants, S1 treatment caused 4.71%, 13.64%, 9.26% and 12.64% increase in the Car, Chl a, Chl b and total Chl content, while S2 caused 17.04%, 20.23%, 15.20% and 19.07% increase in the Car, Chl a, Chl b and total Chl content. However, the difference of Chl a/b was not significant between AM seedlings and non-AM seedlings. Inoculation with AMF can help increase the content of photosynthetic pigments, and then promote the growth and development of *P. polyphylla* var. *yunnanensis*.

Table 1: Effects of AMF on photosynthetic pigment content of seedlings in *Paris polyphylla* var. *yunnanensis*

Treatments	Sites	Car ($mg \cdot g^{-1}$)	Chl a ($mg \cdot g^{-1}$)	Chl b ($mg \cdot g^{-1}$)	Total Chl ($mg \cdot g^{-1}$)	Chl *a*/*b*
S1	Wanzhou	171.435 ± 0.018a	1.754 ± 0.023a	0.553 ± 0.022a	2.306 ± 0.023a	3.175 ± 0.000a
	Anshun	194.704 ± 0.110a	1.872 ± 0.112a	0.555 ± 0.097a	2.427 ± 0.057a	3.373 ± 0.107a
	Baoshan	139.430 ± 0.215a	1.483 ± 0.161a	0.460 ± 0.103a	1.943 ± 0.087a	3.225 ± 0.034a
S2	Wanzhou	179.267 ± 0.084a	1.893 ± 0.071a	0.589 ± 0.090a	2.482 ± 0.075a	3.220 ± 0.022a
	Anshun	193.627 ± 0.118a	1.825 ± 0.091a	0.553 ± 0.094a	2.378 ± 0.091a	3.302 ± 0.021a
	Baoshan	155.851 ± 0.106a	1.569 ± 0.073a	0.485 ± 0.102a	2.054 ± 0.078a	3.247 ± 0.060a
CK	Wanzhou	134.891 ± 0.006b	1.364 ± 0.033b	0.480 ± 0.154a	1.741 ± 0.067b	3.438 ± 0.072a
	Anshun	160.051 ± 0.075b	1.384 ± 0.189b	0.415 ± 0.161b	1.799 ± 0.182b	3.318 ± 0.051a
	Baoshan	133.162 ± 0.079a	1.305 ± 0.154a	0.421 ± 0.154a	1.725 ± 0.028a	3.095 ± 0.079a

Note: All data are means ± SE ($n = 4$). Different letters indicate significant differences ($p < 0.05$) determined by Duncan's multiple range test. The same as below. S1 means the inoculation with *Scutellospora calospora*, *Cetraspora pellucida*, *Racocetra coralloidea* and *Racocetra fulgida*; S2 means the inoculation with *Scutellospora calospora*, *Cetraspora pellucida*, *Gigaspora margarita*, *Gigaspora gigantea*, *Septoglomus deserticola* and *Claroideoglomus claroideum*; Ck means the inoculation without any arbuscular mycorrhizal fungi.

3.4 Effects of AMF on the Photosynthetic Parameters in Paris polyphylla var. yunnanensis

Mycorrhizal fungal treatments significantly improved P_N in comparison to the non-mycorrhizal fungal treated plants (Tab. 2). S1 treatment dramatically increased P_N by 134.91% in Wanzhou, 24.17% in Anshun and 108.80% Baoshan compared with control, while S2 treatment, they were by 127.70%, 15.99% and 77.73%, respectively. In addition, application of S1 and S2 fungus combination improved other photosynthetic parameters in *P. polyphylla* var. *yunnanensis* leaves compared with the plants under natural environment, such as E, C_i, G_s and water use efficiency (WUE).

3.5 Effects of AMF on the Protective Enzymes Activities in Paris polyphylla var. yunnanensis

CAT, POD and SOD activities were increased in inoculated *P. polyphylla* var. *yunnanensis* plants by 8.96–14.88%, 37.79–215.32% and 52.03–98.34% in S1 treatment, and by 4.68–13.66%, 84.66–165.51%, and 18.89–33.10% in S2 treatment, respectively, compared to the non-inoculated plants (Fig. 3).Hence, the inoculation of AMF was beneficial to increase the protective enzyme activity of *P. polyphylla* var. *yunnanensis* leaves.

Table 2: Effects of AMF on photosynthetic parameters of seedlings in *Paris polyphylla* var. *yunnanensis*

Treatments	Sites	P_N (μmol·m^{-2}·s^{-1})	T_r (mmol·m^{-2}·s^{-1})	WUE (μmol·mol^{-1})	G_s (mmol·m^{-2}·s^{-1})	L_s	C_i (μmol·mol^{-1})
S1	Wanzhou	3.782 ± 0.044a	0.375 ± 0.011a	10.078 ± 0.131a	28.571 ± 0.027a	0.013 ± 0.156a	378.783 ± 0.002a
	Anshun	4.799 ± 0.052a	1.479 ± 0.034a	3.244 ± 0.017a	123.285 ± 0.011a	0.213 ± 0.020a	315.483 ± 0.003a
	Baoshan	4.153 ± 0.019a	1.423 ± 0.083a	2.919 ± 0.064a	493.138 ± 0.001a	0.020 ± 0.175a	407.968 ± 0.001a
S2	Wanzhou	3.666 ± 0.052a	0.919 ± 0.112b	3.987 ± 0.003b	47.718 ± 0.014b	0.017 ± 0.594a	382.410 ± 0.007a
	Anshun	4.483 ± 0.048ab	1.535 ± 0.036a	2.920 ± 0.012b	140.766 ± 0.016b	0.121 ± 0.011b	350.257 ± 0.004b
	Baoshan	3.535 ± 0.051b	1.246 ± 0.090a	2.837 ± 0.039a	404.671 ± 0.006b	0.045 ± 0.717a	392.152 ± 0.029a
CK	Wanzhou	1.610 ± 0.097b	0.416 ± 0.058a	3.871 ± 0.046b	24.742 ± 0.044c	0.174 ± 0.013b	324.829 ± 0.004b
	Anshun	3.865 ± 0.045b	0.893 ± 0.157b	2.373 ± 0.028c	150.185 ± 0.002c	0.297 ± 0.021c	274.802 ± 0.009c
	Baoshan	1.989 ± 0.009c	0.716 ± 0.101b	2.778 ± 0.110a	101.417 ± 0.007c	0.092 ± 0.056a	364.158 ± 0.006b

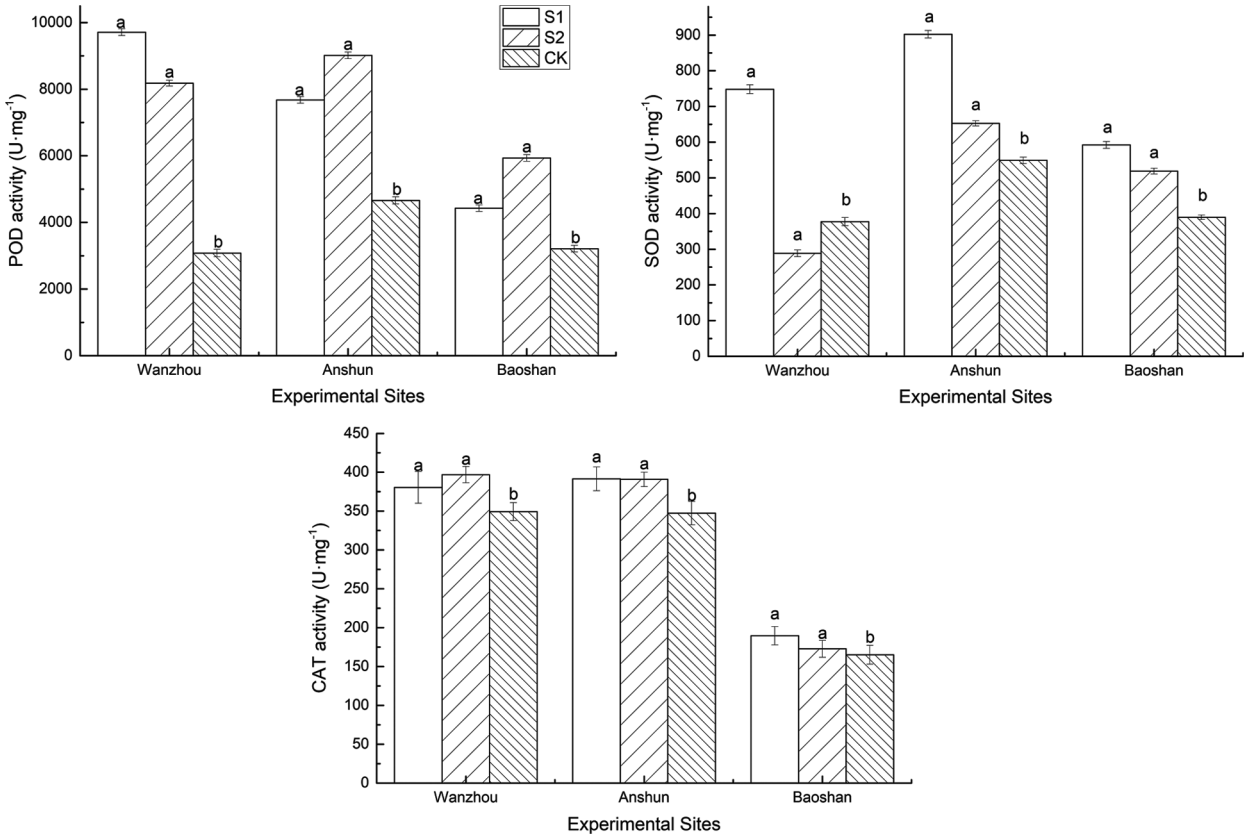

Figure 3: Effects of AMF on CAT, POD and SOD activities of seedlings in *Paris polyphylla* var. *yunnanensis*

3.6 Effects of AMF on the MDA, Soluble Sugar and Soluble Protein Content in Paris polyphylla var. yunnanensis

AMF inoculation induced dramatic reduction of MDA (an indicator of lipid peroxidation) (Fig. 4). Exogenous inoculation of AMF significantly reduced the MDA content, thereby reducing the degree of membrane lipid peroxidation.

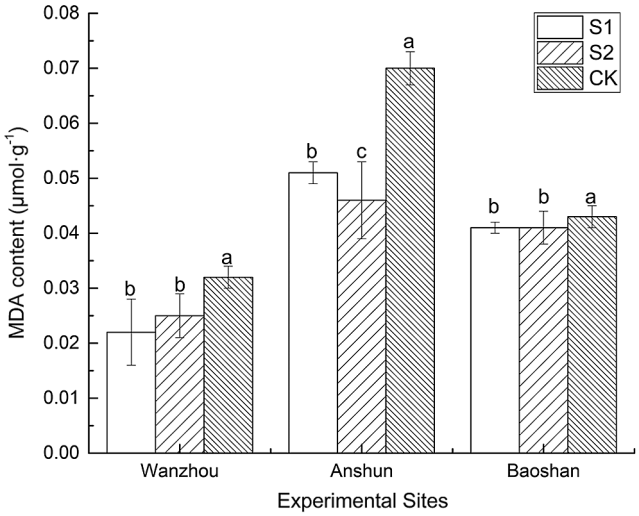

Figure 4: Effects of AMF on MAD content of seedlings in *Paris polyphylla* var. *yunnanensis*

The soluble sugar and soluble protein contents in *P. polyphylla* var. *yunnanensis* were significantly increased under inoculation AMF environment (Figs. 5a, 5b). Compared to the CK group, the soluble sugar content in Wanzhou, Anshun and Baoshan was increased by 25.91%, 85.24% and 123.93% in the S1 group, and by 9.91%, 60.22% and 95.26% in the S2 group, respectively. The soluble protein content in Wanzhou, Anshun and Baoshan was increased by 22.70%, 15.36% and 4.26% in the S1 group, and by 22.70%, 15.36% and 4.26% in the S2 group, respectively, compared with the CK group plants.

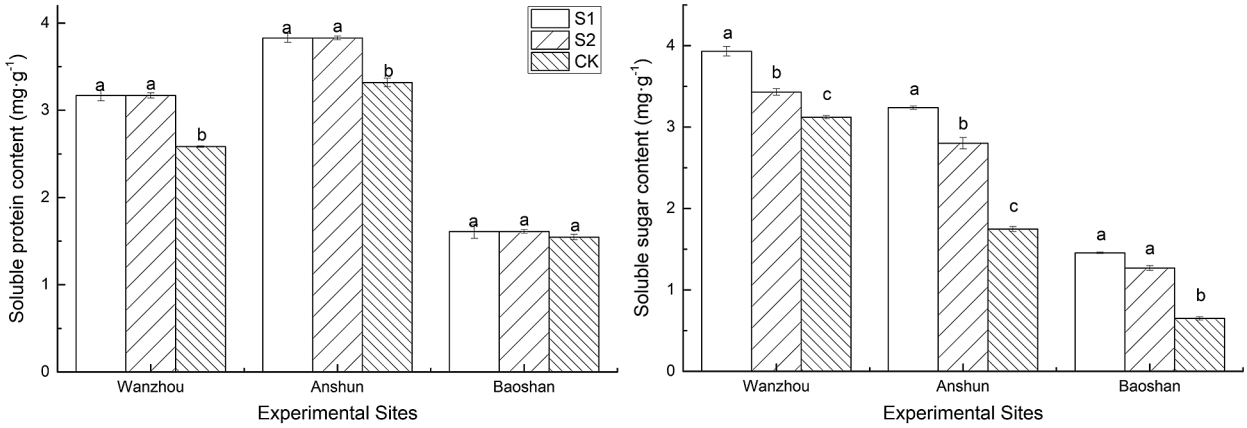

Figure 5: Effects of AMF on soluble sugar and soluble protein content of seedlings in *Paris polyphylla* var. *yunnanensis*

3.7 Effects of AMF on the Biomass in Paris polyphylla var. yunnanensis

Fresh weight and dry weight were significantly higher in mycorrhizal than non-mycorrhizal plants (Tab. 3). In Wanzhou, S1 treatment caused 48.74% and 55.96% significant ($p < 0.05$) increase in the above indicators, while S2 treatment, they were 38.98% and 32.46% compared to non-AM seedlings, respectively. In Anshun, compared with the controls, S1 treatment caused 22.31% and 29.07% obvious ($p < 0.05$) enhancement in the above indicators, while S2 treatment, they were 76.52% and 130.19%, respectively. In Baoshan, compared with the control plants, S1 treatment caused 50.42%

and 45.10% increase in the above indicators, while S2 pretreatment they were enhanced by 28.31% and 51.98%, respectively.

Table 3: Effects of AMF on rhizomes biomass and rhizome drying rate of seedlings in *Paris polyphylla* var. *yunnanensis*

Treatments	Sites	Fresh weight (g)	Dry weight (g)	Drying rate (%)
S1	Wanzhou	12.188 ± 0.007a	4.445 ± 0.003a	36.138 ± 0.001a
	Anshun	5.657 ± 0.015b	2.069 ± 0.006b	36.592 ± 0.001b
	Baoshan	5.254 ± 0.020a	1.541 ± 0.006a	29.989 ± 0.001c
S2	Wanzhou	11.388 ± 0.008b	3.775 ± 0.003b	31.974 ± 0.001c
	Anshun	8.164 ± 0.008a	3.690 ± 0.004a	45.367 ± 0.001a
	Baoshan	4.482 ± 0.019b	1.614 ± 0.007a	36.264 ± 0.001a
CK	Wanzhou	8.194 ± 0.011c	2.850 ± 0.004c	34.615 ± 0.001b
	Anshun	4.625 ± 0.020c	1.603 ± 0.007c	34.705 ± 0.001c
	Baoshan	3.493 ± 0.030c	1.062 ± 0.009b	32.089 ± 0.001b

3.8 Effects of AMF on the Polyphyllin Yield and Content in Paris polyphylla var. yunnanensis

Compared with the non-AMF-inoculated plants under natural environment alone, S1 and S2 treatments effectively improved the yield of polyphyllin by 29.89–135.39% (Fig. 6). The yield of polyphyllin reached maximum values when plants were inoculated by S2 treatment in Anshun.

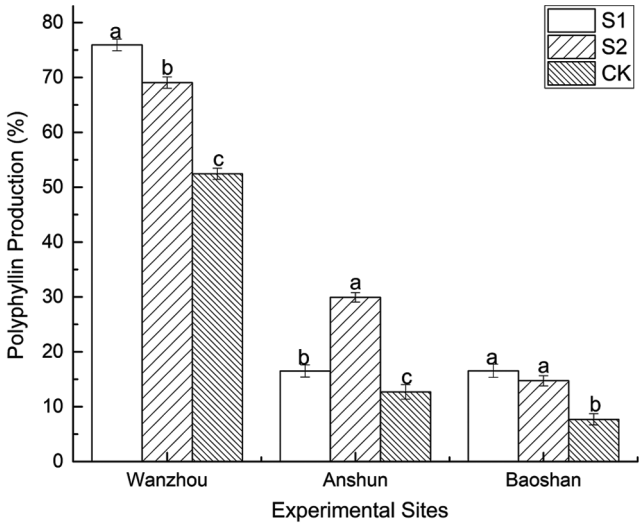

Figure 6: Effects of AMF on polyphyllin production of seedlings in *Paris polyphylla* var. *yunnanensis*

The types of *P. polyphylla* var. *yunnanensis* polyphyllin vary with the organs of *P. polyphylla* var. *yunnanensis*. A marked increase in Polyphyllin I, Polyphyllin II, Polyphyllin VI, Polyphyllin VII and total polyphyllin content was observed in AMF-treated seedlings of different parts (Tab. 4). In Wanzhou, total polyphyllin content was enhanced by 13.39%~1105.58% in S1 treatment and by 11.54%~774.26% in S2 treatment. In Anshun, total polyphyllin content was enhanced by 15.21%~55.61% in S1 treatment

and by 9.42%~109.68% in S2 treatment. In Baoshun, total polyphyllin content was enhanced by 27.56% ~43.86% in S1 treatment and by 11.22%~32.00% in S2 treatment. As a result, the plants supplemented with AMF could improve medicinal quality, especially four kinds of polyphyllin and total polyphyllin, in *P. polyphylla* var. *yunnanensis*.

4 Discussion

AMF are extremely sensitive to the environment, and its infection status is affected by factors such as crop type, soil fertility, climatic conditions and agricultural measures [7]. In this experiment, the infection rate of *P. polyphylla* var. *yunnanensis* seedlings inoculated with AMF was 62.96%~82.72%, indicating that the seedling stage of *P. polyphylla* var. *yunnanensis* was the best period for inoculation of exogenous AMF. Compared with the CK group, SDH and ALP activities were significant enhanced by AMF inoculation, indicating that three experimental sites (Wanzhou, Anshun, and Baoshan) could form good mycorrhizas in roots of *P. polyphylla* var. *yunnanensis*.

Recent studies indicated that AMF increased chlorophyll content, P_N and *G*s, improved photosynthetic performance of plants, and enhanced growth and development [9,15]. CAT, POD and SOD as the key enzymes for scavenging free radicals in plants, can play an important role in maintaining the balance of oxygen metabolism. Existing studies have shown that the increase of protective enzyme activity, soluble sugar and soluble protein content is conducive to enhancing plant stress resistance, including salinity [10], drought [11,28], and temperature stress [15,24]. Compared with the non-AMF plants, the Chl a, Chl b, and total Chl contents in the leaves of *P. polyphylla* var. *yunnanensis* were increased under mycorrhization. The photosynthesis of *P. polyphylla* var. *yunnanensis* leaves inoculated with different AMF mixed treatments in the field was different. Compared with the CK group, the photosynthesis of treatment groups S1 and S2 were enhanced. Among the three field planting sites, *P. polyphylla* var. *yunnanensis* in Anshun and Wanzhou had better photosynthetic capacity. In addition, AMF inoculation dramatically enhanced SOD, POD, and CAT activities of *P. polyphylla* var. *yunnanensis*, almostly dependent on experimental sites, indicating that field application of AMF could improve antioxidant capacity of host plants, as seen by a low MDA content in AMF-inoculated plants. Similar results were reported by Zhang et al. [9] in trifoliate orange colonizaed by *Funneliformis mosseae*. Hence, the field test of *P. polyphylla* var. *yunnanensis* rhizome inoculated with AMF was relatively successful.

The formation of mycorrhiza not only stimulated the growth of plants, but also increased the accumulation of related active ingredients. Previous studies have showed that AMF greatly improved the medicinal quality in traditional Chinese medicine including *Atractylodes lancea* [31], *Medicago truncatula* [32], *M. sativa* [33] and so on. Polyphyllin is considered to be one of the main active ingredients in Rhizoma *Paridis* (“Chong-lou” in Chinses), the dried rhizomes of *P. polyphylla* var. *yunnanensis*, which has various activities, such as detoxification and pain relief (Chinese Pharmacopoeia Committee, 2015). The two mixed microbial agents used in this experiment increased the content of polyphyllin. I guess that AMF inoculation potentially stimulated activity of plant second metabolism, thus, promoting the polyphyllin accumulation in *P. polyphylla* var. *yunnanensis*, which still needs to be further studied. The increase of polyphyllin indicates that establishing dominant mycorrhizal fungus populations in the field and accelerating mycorrhizal infection is effective ways to improve its production. According to the records in the latest edition of the Chinese Pharmacopoeia, the amount of 4 kinds of polyphyllin content not be less than 0.60%. In this experiment, the content of polyphyllin in the old and new rhizomes of *P. polyphylla* var. *yunnanensis* in all treatment groups reached the standard of medicinal materials.

Table 4: Effects of AMF on the content of polyphyllin from new rhizome, old rhizome and fibrous roots of seedlings in *Paris polyphylla* var. *yunnanensis*

Treatments	Sites	Parts	Polyphyllin I (%)	Polyphyllin II (%)	Polyphyllin VI (%)	Polyphyllin VII (%)	The ratio of polyphyllin I-II-VI-VII	Total polyphyllin (%)
S1	Wanzhou	New rhizome	8.363 ± 0.005a	4.141 ± 0.045a	2.905 ± 0.042a	2.192 ± 0.060a	1.000:0.495:0.347:0.262	17.601 ± 0.014a
		Old rhizome	9.379 ± 0.005a	2.303 ± 0.017a	3.639 ± 0.024a	3.246 ± 0.057a	1.000:0.246:0.388:0.892	18.567 ± 0.001a
		Fibril root	2.000 ± 0.061a	2.127 ± 0.019a	10.123 ± 0.012a	12.540 ± 0.005a	1.000:1.063:5.061:6.270	26.788 ± 0.001a
	Anshun	New rhizome	1.556 ± 0.048a	3.187 ± 0.036a	2.588 ± 0.014a	0.817 ± 0.163a	1.000:2.048:1.663:0.525	8.149 ± 0.011a
		Old rhizome	1.354 ± 0.003a	2.378 ± 0.018a	3.580 ± 0.008a	1.319 ± 0.014a	1.000:1.756:2.644:0.368	8.630 ± 0.007a
		Fibril root	0.020 ± 0.013a	0.304 ± 0.010a	2.279 ± 0.031a	0.457 ± 0.133a	1.000:14.922:111.725:22.382	3.061 ± 0.161a
	Baoshan	New rhizome	1.562 ± 0.008a	2.596 ± 0.004a	2.970 ± 0.012a	0.892 ± 0.017a	1.000:1.663:1.902:0.571	8.020 ± 0.002a
		Old rhizome	1.430 ± 0.015a	2.211 ± 0.008a	3.816 ± 0.003a	1.219 ± 0.012a	1.000:1.546:2.669:0.319	8.780 ± 0.005a
		Fibril root	0.011 ± 0.003a	0.686 ± 0.035a	2.736 ± 0.030a	0.025 ± 0.003a	1.000:64.344:256.469:2.313	3.457 ± 0.071a
S2	Wanzhou	New rhizome	8.717 ± 0.014b	3.121 ± 0.009b	2.618 ± 0.046a	2.858 ± 0.022b	1.000:0.358:0.300:0.328	17.314 ± 0.016a
		Old rhizome	7.335 ± 0.000b	5.320 ± 0.024b	3.384 ± 0.008b	2.888 ± 0.007b	1.000:0.725:0.461:0.853	18.928 ± 0.009b
		Fibril root	2.170 ± 0.047b	5.682 ± 0.021b	3.807 ± 0.183b	12.442 ± 0.007a	1.000:2.618:1.754:5.733	19.426 ± 0.037b
	Anshun	New rhizome	1.358 ± 0.027a	3.252 ± 0.022a	2.189 ± 0.058b	0.939 ± 0.060a	1.000:2.395:1.612:0.692	7.739 ± 0.019b
		Old rhizome	0.766 ± 0.083b	2.895 ± 0.005b	2.426 ± 0.046b	1.900 ± 0.004b	1.000:3.778:3.166:0.783	7.988 ± 0.009b
		Fibril root	0.039 ± 0.004a	0.832 ± 0.011b	2.930 ± 0.034b	0.732 ± 0.055b	1.000:21.197:74.650:18.643	4.659 ± 0.028
	Baoshan	New rhizome	1.587 ± 0.025a	2.273 ± 0.051a	3.025 ± 0.011a	0.951 ± 0.061a	1.000:1.432:1.906:0.599	7.837 ± 0.017a
		Old rhizome	1.439 ± 0.025a	1.924 ± 0.060b	2.865 ± 0.019b	0.967 ± 0.042b	1.000:1.337:1.991:0.337	7.267 ± 0.024b
		Fibril root	0.018 ± 0.004a	0.615 ± 0.004b	2.479 ± 0.047b	0.060 ± 0.014a	1.000:34.167:137.722:3.306	3.172 ± 0.069b
CK	Wanzhou	New rhizome	8.235 ± 0.011a	3.151 ± 0.064b	1.945 ± 0.041b	2.192 ± 0.060a	1.000:0.383:0.236:0.266	15.523 ± 0.010b
		Old rhizome	2.648 ± 0.026c	2.245 ± 0.023a	3.657 ± 0.002a	1.683 ± 0.016c	1.000:0.848:1.381:0.460	10.234 ± 0.009c
		Fibril root	0.021 ± 0.013c	0.199 ± 0.136c	1.633 ± 0.008c	0.369 ± 0.075b	1.000:9.458:77.762:17.583	2.222 ± 0.032c
	Anshun	New rhizome	1.513 ± 0.036a	2.467 ± 0.044b	2.472 ± 0.018a	0.622 ± 0.025a	1.000:1.630:1.634:0.411	7.073 ± 0.004c
		Old rhizome	0.715 ± 0.022b	2.074 ± 0.002c	2.160 ± 0.011c	0.599 ± 0.005c	1.000:2.901:3.021:0.277	5.546 ± 0.007c
		Fibril root	0.021 ± 0.003a	0.199 ± 0.010c	1.633 ± 0.048c	0.369 ± 0.024a	1.000:9.458:77.762:17.583	2.222 ± 0.085c
	Baoshan	New rhizome	1.149 ± 0.002b	1.738 ± 0.113b	2.502 ± 0.057b	0.897 ± 0.008a	1.000:1.512:2.177:0.781	6.287 ± 0.007b
		Old rhizome	0.836 ± 0.107b	1.335 ± 0.029c	2.953 ± 0.018b	1.411 ± 0.091a	1.000:1.598:3.534:0.478	6.534 ± 0.047b
		Fibril root	0.015 ± 0.003a	0.359 ± 0.003c	1.977 ± 0.034c	0.053 ± 0.006a	1.000:24.759:136.310:3.621	2.403 ± 0.028c

In summary, different experimental treatments had different influences on the growth and development and medicine quality of *P. polyphylla* var. *yunnanensis*. Among the three field test sites, Wanzhou had the better field cultivation effect. Therefore, it is possible to improve the medicinal quality of *P. polyphylla* var. *yunnanensis* from small-scale laboratory experiments to inoculation of exogenous AMF in the field environment, which may bring great economic benefits.

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