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Mycorrhizal Effects on Active Components and Associated Gene Expressions in Leaves of *Polygonum cuspidatum* under P Stress

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Abstract: Arbuscular mycorrhizal fungi (AMF) participate in the process of plant secondary metabolism and thus affect the production of secondary metabolites. However, it is not clear whether and how AMF affect the growth and secondary metabolites of *Polygonum cuspidatum*, a medicinal plant rich in resveratrol and polygonin, under different phosphorus (P) levels. This study was performed to analyze the effects of *Glomus mosseae* on the growth, leaf gas exchange, P concentration, active ingredient concentrations, and expressions of associated genes of *P. cuspidatum* under P-deficient (0 mol/L P) and P-sufficient (0.2 mol/L P) conditions. The root mycorrhizal colonization rate of inoculated plants was 62.53–73.18%. *G. mosseae* improved shoot and root biomass as well as leaf P levels to some extent, but the improvement was more prominent under P-sufficient than P-deficient conditions. The fungal colonization also significantly increased leaf photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration, which was more prominent under P-deficient rather than P-sufficient conditions. P addition promoted the concentration of active medicinal components in leaves, especially in uninoculated plants. *G. mosseae* distinctly raised leaf chrysophanol, emodin, polydatin, and resveratrol concentrations, which was more prominent under P-deficient conditions. However, physcion was raised by *G. mosseae* only under P-sufficient conditions. AMF and P addition up-regulated expressions of *PcCRS1*, along with the up-regulation of *PcRS11* by P addition and *PcRS11* and *PcSTS* by AMF under P-sufficient conditions. It is concluded that an adequate P fertilizer and AMF facilitate the production of active medicinal components in *P. cuspidatum*, associated with expressions of associated genes such as *PcCRS1*.

Keywords: *Glomus mosseae*; mycorrhiza; P fertilizer; resveratrol; secondary metabolites

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1. Introduction

Phosphorus (P) plays an indispensable role in plant growth, as demonstrated by it not only promoting chlorophyll synthesis, but also by it participating in electron transfer, energy metabolism, photosynthetic phosphorylation, and carbon assimilation [1–4]. However, P deficiency often occurs during plant growth [5] because of the poor solubility and slow diffusion of phosphate in the soil, as well as its easy fixation by iron oxide and alumina in the soil, resulting in very low P levels [6]. Therefore, it is urgent to promote plant growth under low P soil conditions.

Soil arbuscular mycorrhizal fungi (AMF) can form a mutually beneficial arbuscular mycorrhizal symbiosis with about 80% of terrestrial plant roots [7], where it helps roots to absorb nutrients and water and to coordinate physiological metabolisms, among which the improvement of P nutrition and secondary metabolism is particularly prominent [8,9]. The supply of P fertilizer improved the yield and quality of essential oils in *Foeniculum vulgare* plants [10]. Soil P levels affected the role of mycorrhizal symbiosis on plants to some extent [11]. However, AMF, but not P fertilizer, promoted essential oil levels in oregano plants [12]. The interactive effect of AMF with P fertilizer was confirmed by Tan et al. [13], who reported that the combination of low P levels and *Glomus mosseae* inoculation raised artemisinin accumulation in *Artemisia annua*, while the combination of high P levels and *G. mosseae* inoculation showed inhibitory effects. On the contrary, in *Anadenanthera colubrina*, AMF had positive benefits on the levels of various secondary metabolites such as total phenols and total flavonoids, even under high P supply conditions [14]. Therefore, the interaction between P and AMF in regulating secondary metabolites in medicinal plants seems to be very complex and needs to be studied in depth.

Polygonum cuspidatum Sieb. Et Zucc is a perennial herb that is of interest because of its active substances [15]. Among its secondary metabolites, resveratrol as a naturally polyphenolic antioxidant [16], is found in a relatively high level in *P. cuspidatum* among many plants [17]. Resveratrol synthase (RS), stilbene synthase (STS), resveratrol backbone stilbene synthase (PCRS), and polyketide stilbene synthase1 (CRS) are involved in resveratrol synthesis in *P. cuspidatum* [18,19]. STS is only present in certain plants for resveratrol synthesis [20]. In addition, other secondary metabolites of *P. cuspidatum* such as rhodopsin, polydatin, physcion, and emodin exhibit various medicinal properties, and are widely used in pharmaceutical and nutraceutical industries [21]. Therefore, it is important to improve medicinal component levels of *P. cuspidatum*. However, most of *P. cuspidatum* is grown in mountainous areas of China, where soil P levels are relatively low [22,23]. It is unclear whether and how AMF inoculation and P interact to affect plant growth and medicinal component levels in *P. cuspidatum*.

Since both AMF and P fertilizer supply can promote the level of secondary metabolites in *P. cuspidatum*, it is unclear whether this benefit is more dependent on AMF or P fertilizer and whether it depends on the expression of related genes in the pathway of secondary metabolites caused by AMF. The objective of this study was to analyze the effects of AMF inoculation on leaf gas exchange, plant growth, active component levels, and expressions of resveratrol-associated enzyme genes in leaves of *P. cuspidatum* under low and appropriate P conditions, in anticipation of revealing the benefits and potential mechanisms of P and AMF in promoting the active medicinal components of *P. cuspidatum*.

2. Materials and Methods

2.1. Experimental Design

The study consisted of the following factors: (i) P treatments, including appropriate P level (0.2 mol/L P) (Ap) and low P level (0 mol/L P) (Lp); and (ii) inoculation treatments, including *G. mosseae* inoculation (G^+) and non-inoculation (G^-). A total of four treatments (ApG^+, ApG^-, LpG^+, and LpG^-) were performed in this experiment, each with eight replicates, in a completely randomized block arrangement.

2.2. Experimental Set-Up

P. cuspidatum with four leaves growing in autoclaved soil was transplanted into a plastic pot (height: 18 cm; upper diameter: 21 cm; base diameter: 12 cm) where 2.05 kg of sulfuric acid-eluting sand was pre-filled. For the G^+ treatment, 150 g of *G. mosseae* inoculum was supplied per pot, while the G^- treatment received 150 g of autoclaved fungal inoculum per pot, accompanied by 2 mL of 30 μm filtered solution of the same inoculum. The *G. mosseae* strain, preserved in our laboratory, was isolated from the rhizosphere of *Incarvillea younghusbandii*, identified in morphological levels, and trapped with white clover as the host for 12 weeks. The fungal inoculum thus consisted of fungal-colonized root segments,

spores, and hyphae, in which the number of spores was 19 spore/g. After inoculation treatments, these seedlings were acclimated indoors for 4 days before being placed in an environmentally controlled greenhouse, in which the environmental conditions were as described by Sun et al. [24]. Plants were kept watered with distilled water at the rate of 100 mL/pot per day. Three weeks later, P treatment was started, using Hoagland nutrient solution adjusted for P levels. Substrate P levels were set at 0 mol/L and 0.2 mol/L in 3-day intervals at 70 mL of nutrient solution per pot, referring to the results of Song et al. [25]. For a total of 17 times, different strengths of P solution were administered. The potted plants were maintained for 12 weeks and then harvested.

2.3. Variable Measurement

Leaf gas exchange (photosynthetic rate, transpiration rate, intercellular CO_2 concentration, and stomatal conductance) was measured using a Li-6400 (LI-COR Inc., Lincoln, NE, USA) portable photosynthesis system from 9:00 to 10:00 am on the harvested day of *P. cuspidatum* (a sunny day), with the top 3rd of the leaf [26]. Then, the plants were removed from plastic pots and divided into shoots and roots. A small number of leaves were treated with liquid nitrogen and stored at −75 °C for gene expression assay. Twelve root segments of approximately 1-cm length were cut from the root of each plant and stained using 0.05% (*w*/*v*) of trypan blue in lactic acid phenol solutions [27]. Root mycorrhizas were observed microscopically, and the degree of AMF colonization was estimated as the percentage of the length of the AMF-colonized root segments versus the total length of the observed root segments. The rest of the material was treated at 105 °C for 3 min and then at 75 °C for drying to constant weight [28]. The respective dry weight was determined. Leaf P concentrations were determined using a colorimetric method outlined by Cavell [29] by grinding leaves, passing them through a 2 mm sieve, and then digesting them with H_2SO_4-H_2O_2.

Total chlorophyll concentration in leaves was calculated by the colorimetric method, based on the extraction with 80% acetone solution [30]. Total soluble protein concentration in leaves was measured by the protocol of Bradford [31], using bovine serum albumin as a standard.

A 0.1 g dried leaf sample ($\Phi < 2$ mm) was incubated with 10 mL of 80% methanol for half an hour under sonicated conditions, and centrifuged at 4500× *g* for 10 min. The supernatant was collected as the solution to be measured and analyzed on a HPLC (LC-20A, Shimadzu, Kyoto, Japan), where the chromatographic conditions were an Agela Venusil XBP C18(L) column (4.6 × 250 mm, 5 μm) with acetonitrile (A) and 0.1% formic acid (B) as the mobile phase, a 1.0 mL/min flow rate, a 10 μL injection volume, a detection wavelength of 290 nm, and a column temperature of 75 °C.

Total RNA of the leaves was extracted using a Quick RNA isolation kit (ZH120, Huayueyang, Beijing, China) and then reversely transcribed into cDNA as per a TRUE 1st Strand cDNA Synthesis Kit with gDNA Eraser kit (PC5402, Aidlad, Beijing, China). Four related genes in the resveratrol synthesis pathway including *RS*, *STS*, *PCRS11*, and *CRS1* were selected. Their specific primers were designed by Primer premier 5.0 and were shown in Table 1. qRT-PCR assays were performed as per the method of Sun et al. [23], combined with 2× AceQ qPCR SYBR Green Master Mix (Aidlab, Beijing, China). Actin was used as an internal reference control. Relative gene expression was estimated in terms of a comparative method [32] using LpG^- treatment as the standard.

2.4. Statistical Analysis

The obtained data were evaluated using two-way analysis of variance by SAS® software (9.1.3v), and Duncan's multiple-range tests were performed to compare significant differences among treatments at the 0.05 level.

Table 1. Primer sequences of the genes used in the present study for qRT-PCR.

Genes	Gene ID	Forward Sequence (5′→3′)	Reverse Sequence (5′→3′)
PcCRS1	DQ459350.1	TGAGCGAGTACGGGAATTTG	CCTTCTCCAGTCGTCTTCTTAC
PcRS11	EF117977.1	GATGAGATGATGAAGGCACAAAC	GGAAGTAGAAGTCGGGAAAGTC
PcRS	DQ900615.1	GAGATGACGAAGGCACTAACA	GGAAGTAGAAGTCGGGAAAGTC
PcSTS	EU647245.1	GAAGAGATGATGAAGGCACAAAC	GGAAGTAGAAGTCGGGAAAGTC
PcActin	MK288156.1	TACAATGAGCTTCGGGTTGC	GCTCTTTGCAGTTTCCAGCT

3. Results

3.1. Changes in Root Mycorrhizal Colonization and Biomass Production

No signs of AMF colonization were seen in the roots of uninoculated plants with *G. mosseae* grown in autoclaved substrates, while mycorrhizal colonization was seen in the inoculated roots, where the degree of AMF colonization was 62.53 ± 6.65% under Lp and 73.18 ± 7.76% under Ap.

In addition, compared with Lp treatment, Ap treatment significantly raised shoot and root biomass: it was 29% and 35% higher in uninoculated plants and 71% and 123% higher in inoculated plants, respectively (Figure 1). Inoculation with *G. mosseae* dramatically raised shoot biomass under Lp and Ap by 41% and 32%, respectively, along with 94% higher root biomass under Ap only. P supply and AMF inoculation significantly interacted to influence root biomass (Table 2).

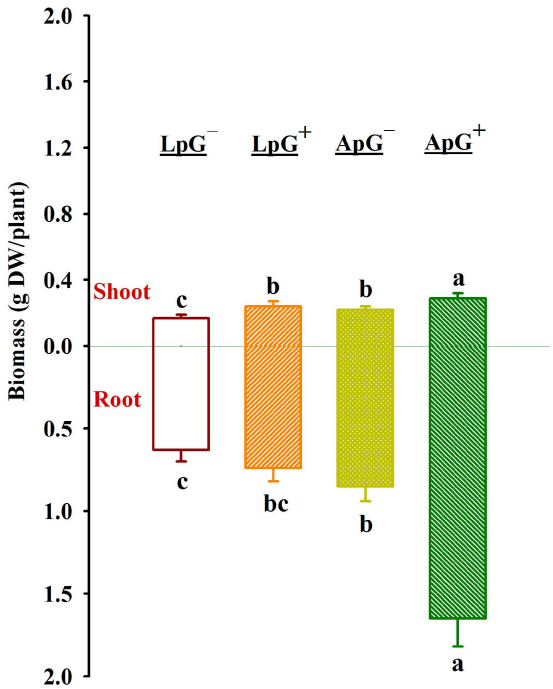

Figure 1. Changes in shoot and root biomass of *Polygonum cuspidatum* inoculated with *Glomus mosseae* in low and approximate P levels. Different letters in the bars indicate significant differences between treatments at the 0.05 level. Abbreviations: LpG^-, plants inoculated without *G. mosseae* in low P levels; LpG^+, plants inoculated with *G. mosseae* in low P levels; ApG^-, plants inoculated without *G. mosseae* in appropriate P levels; ApG^+, plants inoculated with *G. mosseae* in appropriate P levels.

Table 2. Interacted significance of variables.

Variables	P Treatments	AMF Inoculation	Interaction	Variables	P Treatments	AMF Inoculation	Interaction
Shoot biomass	0.0036	0.0010	1.0000	Chrysophanol	0.0323	<0.0001	0.2126
Root biomass	<0.0001	<0.0001	0.0005	Emodin	0.0303	<0.0001	0.1846
P concentrations	0.0004	0.0032	0.3621	Physcion	<0.0001	0.0001	0.0030
Photosynthetic rate	<0.0001	<0.0001	0.2598	Polydatin	<0.0001	0.0030	0.8879
Transpiration rate	<0.0001	<0.0001	0.0007	Resveratrol	0.5037	<0.0001	<0.0001
Stomatal conductance	<0.0001	<0.0001	0.0301	*PcRS*	0.0015	0.0004	<0.0001
Intercellular CO_2 level	0.1799	<0.0001	0.0170	*PcSTS*	0.0752	0.0020	0.2602
Total chlorophyll	<0.0001	0.0006	0.9461	*PcRS11*	<0.0001	0.0121	0.2445
Total soluble protein	<0.0001	0.0070	0.1698	*PcCRS1*	0.0006	0.0049	0.9583

Of all the treatments, co-application of P supply and AMF inoculation presented the best improved effect on shoot and root biomass production.

3.2. Changes in Leaf P Concentrations

P supply significantly increased leaf P concentrations in both inoculated and uninoculated plants (Figure 2). Although a 31% higher P concentration occurred in inoculated plants versus uninoculated plants under Lp, the difference was not significant. AMF inoculation only significantly increased leaf P concentrations under Ap by 37%. Leaf P concentrations were more dependent on AMF inoculation under Ap conditions than under Lp conditions.

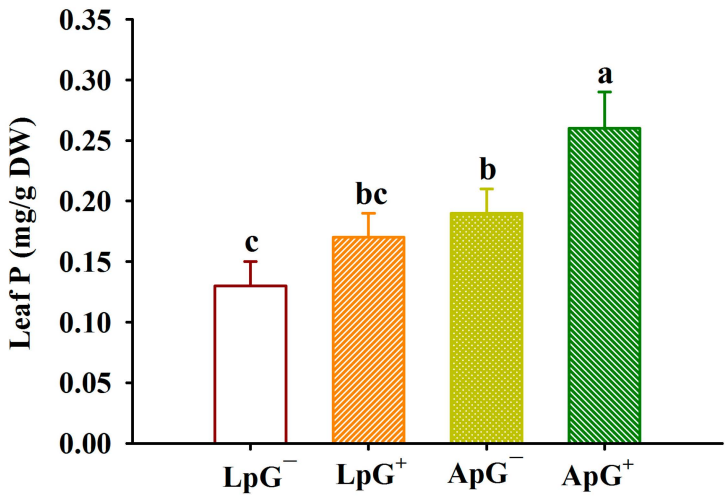

Figure 2. Changes in leaf P concentrations of *Polygonum cuspidatum* inoculated with *Glomus mosseae* in low and approximate P levels. Different letters in the bars indicate significant ($p < 0.05$) differences between treatments. See Figure 1 for abbreviations.

3.3. Changes in Leaf Gas Exchange

The photosynthetic rate and transpiration rate were increased by Ap in both inoculated and uninoculated plants, compared with Lp (Figure 3a,b). In addition, Ap significantly increased stomatal conductance in inoculated plants as well as intercellular CO_2 concentration in uninoculated plants, compared with Lp (Figure 3c,d). Compared to the uninoculated treatment, AMF inoculation significantly increased leaf photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO_2 concentration: 88%, 69%, 100%, and 39% higher under Lp conditions and 67%, 12%, 75%, and 13% higher under Ap conditions, respectively (Figure 3a–d). Transpiration rate, stomatal conductance, and intercellular CO_2 concentration were significantly affected by the interaction of AMF inoculation and P supply (Table 2). In conclusion, AMF inoculation promoted leaf gas exchange, with a higher effect under Lp than under Ap.

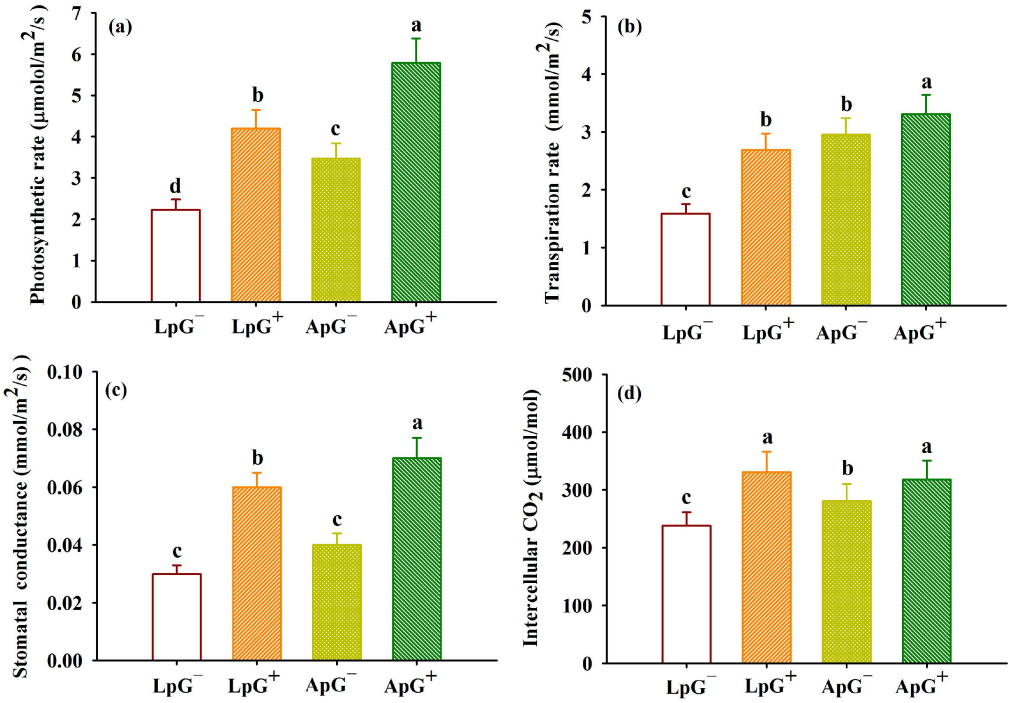

Figure 3. Changes in leaf photosynthetic rate (**a**), transpiration rate (**b**), stomatal conductance (**c**), and intercellular CO_2 concentrations (**d**) of *Polygonum cuspidatum* inoculated with *Glomus mosseae* in low and approximate P levels. Different letters on the bars indicate significant differences between treatments at the 0.05 level. See Figure 1 for abbreviations.

3.4. Changes in Total Chlorophyll and Total Soluble Protein in Leaves

P addition significantly increased leaf total chlorophyll and total soluble protein concentrations, independent of AMF inoculation (Figure 4a,b). In addition, compared to the uninoculated treatment, inoculation with AMF significantly increased leaf total chlorophyll and total soluble protein concentrations: 44% and 44% higher under Lp conditions and 22% and 8% higher under Ap conditions, respectively. P supply and AMF inoculation collectively stimulated leaf total chlorophyll and total soluble protein concentrations.

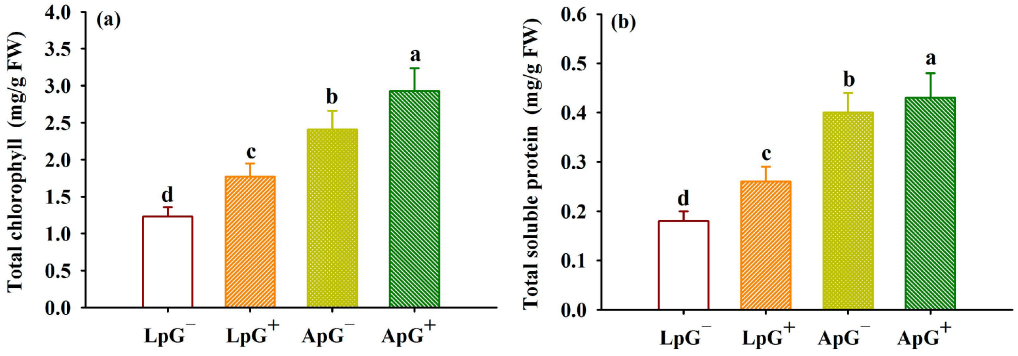

Figure 4. Changes in leaf total chlorophyll (**a**) and total soluble protein (**b**) concentrations of *Polygonum cuspidatum* inoculated with *Glomus mosseae* in low and approximate P levels. Different letters on the bars indicate significant ($p < 0.05$) differences between treatments. See Figure 1 for abbreviations.

3.5. Changes in Active Components in Leaves

A total of five active components were found in leaves of *P. cuspidatum*, with the exception of alloe-emodin, which was below the detection line (Figure 5a,b). Ap treatment significantly increased chrysophanol, emodin, polydatin, and resveratrol levels in leaves of uninoculated plants as well as physcion, polydatin, and resveratrol levels in inoculated

plants, compared with Lp (Figure 6a–e). Under Lp conditions, inoculation with AMF significantly increased chrysophanol, emodin, polydatin, and resveratrol levels in leaves by 180%, 122%, 32%, and 110%, respectively, compared with the uninoculated treatment. Similarly, under Ap conditions, AMF inoculation significantly increased chrysophanol, emodin, physcion, polydatin, and resveratrol levels in leaves by 88%, 64%, 81%, 17%, and 72%, respectively, compared with the uninoculated treatment. In addition, physcion and resveratrol levels were significantly affected by the interaction of AMF and P (Table 2). In short, improved active components in leaves were more dependent on AMF inoculation than P supply.

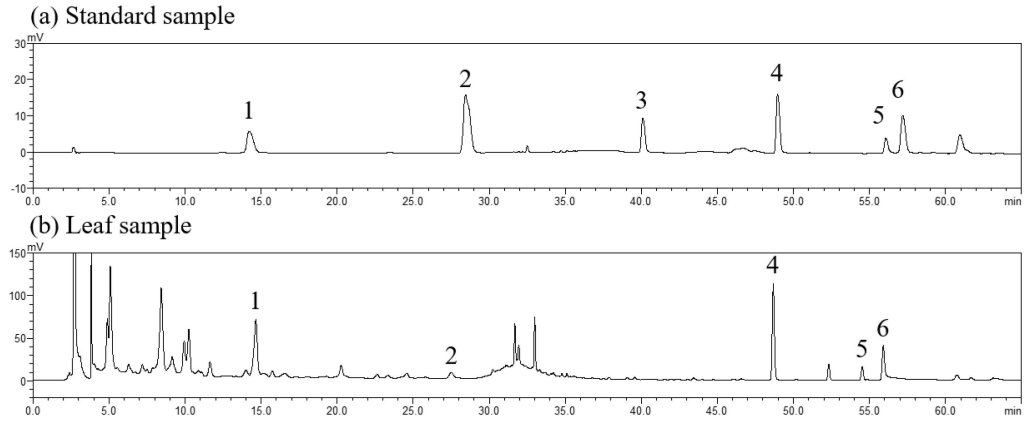

Figure 5. HPLC chromatograms in the determination of standard sample (**a**) and leaf sample (**b**). Among them, 1, 2, 3, 4, 5, and 6 in the figure represent polydatin, resveratrol, aloe-emodin, emodin, chrysophanol, and physcion, respectively.

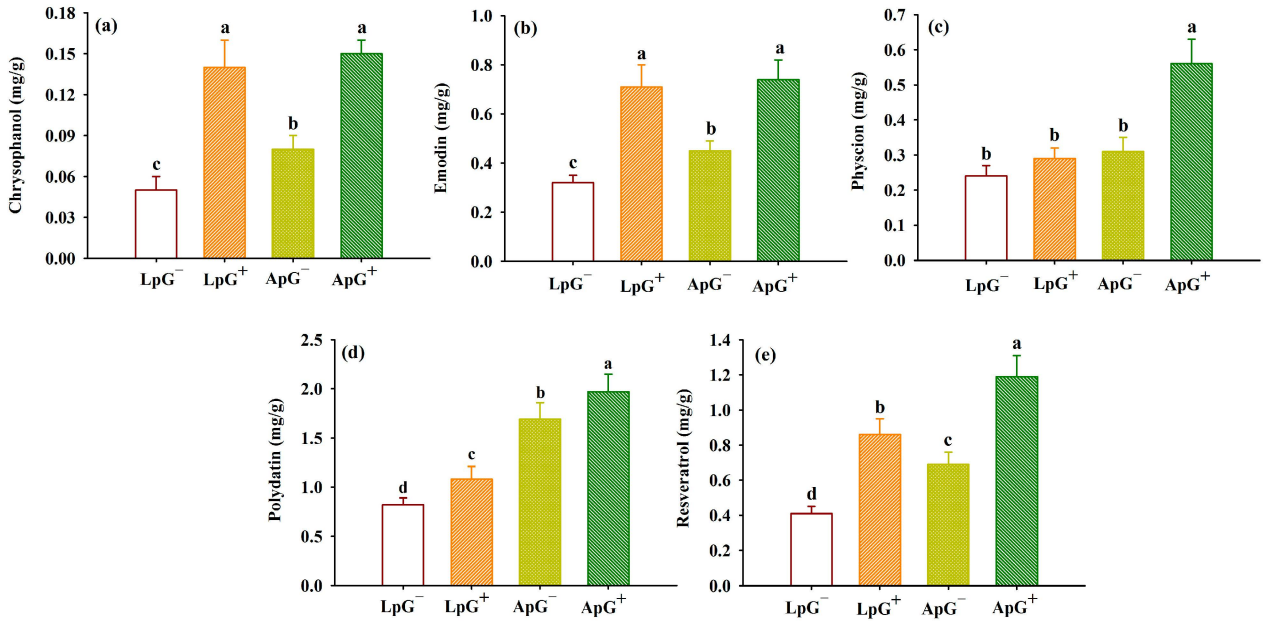

Figure 6. Changes in concentrations of leaf chrysophanol (**a**), emodin (**b**), physcion (**c**), polydatin (**d**), and resveratrol (**e**) of *Polygonum cuspidatum* inoculated with *Glomus mosseae* in low and approximate P levels. Different letters in the bars indicate significant ($p < 0.05$) differences between treatments. See Figure 1 for abbreviations.

3.6. Changes in Expressions of Resveratrol-Associated Enzyme Genes

Ap treatment significantly increased chrysophanol, emodin, polydatin, and resveratrol concentrations in leaves of uninoculated plants as well as physcion, polydatin, and

resveratrol concentrations in inoculated plants, compared with Lp (Figure 7a–d). Under Lp conditions, inoculation with AMF significantly increased chrysophanol, emodin, polydatin, and resveratrol concentrations in leaves by 180%, 122%, 32%, and 110%, respectively, compared with the uninoculated treatment. Similarly, under Ap conditions, AMF inoculation significantly increased chrysophanol, emodin, physcion, polydatin, and resveratrol in leaves by 88%, 64%, 81%, 17%, and 72%, respectively, compared with the uninoculated treatment. In addition, physcion and resveratrol concentrations were significantly affected by the interaction of AMF inoculation and P supply (Table 2).

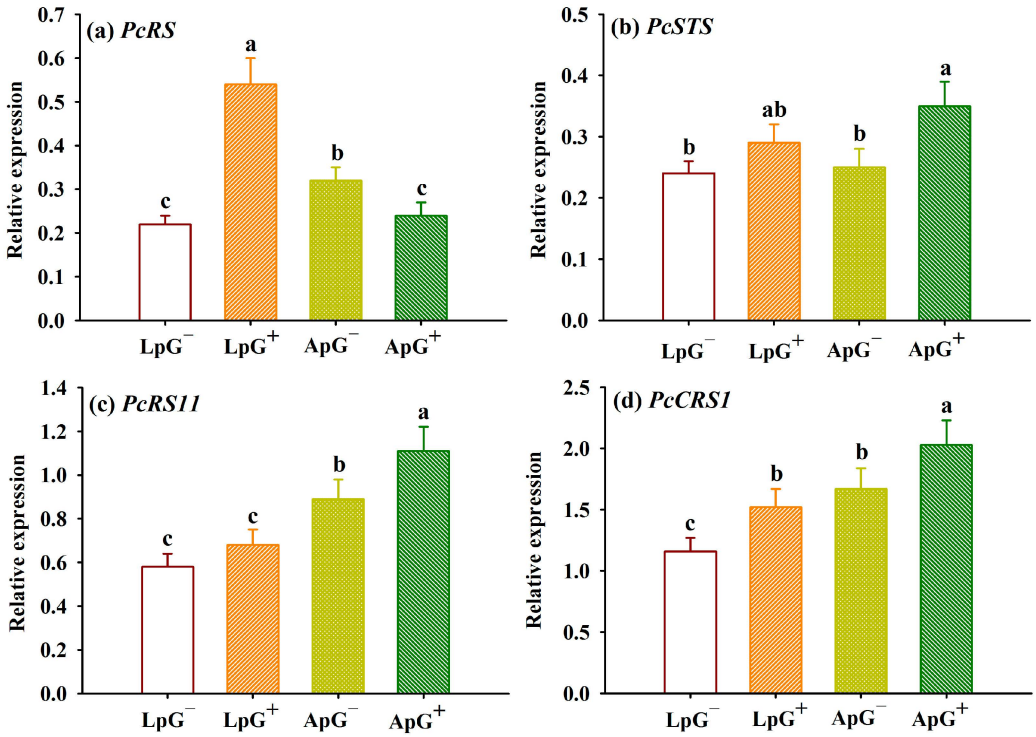

Figure 7. Changes in expressions of leaf *PcRS* (**a**), *PcSTS* (**b**), *PcRS11* (**c**), and *PcCRS1* (**d**) of *Polygonum cuspidatum* inoculated with *Glomus mosseae* in low and approximate P levels. Different letters on the bars indicate significant ($p < 0.05$) differences between treatments. See Figure 1 for abbreviations.

4. Discussion

In this study, it was observed that Ap treatment significantly increased the degree of *G. mosseae* colonization in roots, compared with Lp treatment. Many studies have showed that low P treatment promote the root AMF colonization degree [33]. In fact, there are root hairs and extraradical mycorrhizal hyphae on the root surface, and they have a certain competitive relationship in nutrient absorption [34]. Therefore, the response of mycorrhizal fungi to P may be related to the root hair status of *P. cuspidatum*.

Shoot and root biomass of *P. cuspidatum* was affected by P supply and AMF, and the 0.2 mol/L P level promoted the biomass production of *P. cuspidatum* (Figure 1), indicating that P is an essential mineral element for their growth [35]. On the other hand, *G. mosseae* also improved shoot and root biomass of *P. cuspidatum* (Figure 1), which was more prominent at the 0.2 mol/L P level. However, under the conditions of 0 M P, *G. mosseae* only promoted shoot biomass, but had no significant effect on root biomass, which indicates that *P. cuspidatum* may be more dependent on root hair under the condition of low P. The improvement of plant growth under mycorrhization is due to the fact that mycorrhizal plants showed higher total leaf chlorophyll levels than non-mycorrhizal plants (Figure 4a), regardless of substrate P levels. In fact, AMF can enhance the adaptive ability of host plants to various environmental stresses, including nutrient deficiency, and positively

regulate plant growth [36]. AMF inoculation under drought stress promoted the growth of *Glycyrrhiza uralensis* plants and also increased glycyrrhizinate levels in roots [37,38].

In this study, AMF treatment profoundly promoted the concentration of leaf P under Ap conditions but not under Lp conditions (Figure 2). This is in agreement with the results of Shao et al. [38] who inoculated AMF in tea plants under P deficient conditions. Such results may be because in this study, under Lp conditions, the growth substrate was treated with 0 mM P and almost no P, leading to the fact that the mycorrhizal fungi did not show significant effects, but still increased P by 31%. However, under Ap conditions, mycorrhizal plants promoted P concentrations in *P. cuspidatum*, associated with extraradical mycorrhizal hyphae, released phosphatases, and increased expressions of phosphate transporter genes [39,40].

Both P supply and AMF inoculation could improve leaf gas exchange in *P. cuspidatum*, but the effect of AMF was more prominent (Figure 3). The improved effect of AMF under Lp conditions was higher than that under Ap conditions. Zou et al. [41] found that in *Betula alonoides* AMF colonization distinctly increased leaf gas exchange parameters, thus improving the absorption efficiency of N and P of the host. Similar results are consistent with the improvement of leaf gas exchange variables by inoculation of *Piriformospora indica* (an endophytic fungus) on *P. cuspidatum* [23]. The improvement of P nutrition can enhance the efficiency of plant photosynthate transfer and promote the growth of roots and arbuscular mycorrhizae, which is directly related to the improvement of plant growth [42].

The concentration of active medicinal ingredients is the main criterion to determine the quality of *P. cuspidatum*. In this experiment, P fertilizer could promote the concentration of the active medicinal components in leaves of *P. cuspidatum*, and the more prominent effect was found in uninoculated plants (Figure 6), indicating that mycorrhizal plants were more dependent on AMF than P fertilizer. On the other hand, AMF colonization also dramatically raised chrysophanol, emodin, polydatin, and resveratrol levels, along with a higher improved effect under Lp versus Ap conditions (Figure 6), suggesting the prominent promotion of active component production in *P. cuspidatum* by AMF colonization under low P level conditions. Therefore, AMF inoculation in P-deficient soils was more effective in increasing medicinal component levels. A study by He et al. [43] on *Scutellaria baicalensis* also showed that *G. mosseae* inoculation significantly increased baicalin concentrations, and the best effect was obtained with mycorrhizal fungi under 0.15 g P/kg (a relatively low P level) conditions. Similarly, in *Artemisia annua*, *G. mosseae* promoted artemisinin accumulation under low P level conditions, followed by an inhibitive effect under high P level conditions [13]. In *Paris polyphylla* var. *yunnanensis*, AMF inoculation promoted the concentration of different steroidal saponins in rhizomes, but it depended on the AMF species used [44]. These results suggest that the effect of AMF on a particular secondary metabolite of medicinal plants depends on the combination with AMF species and substrate P levels.

In the process of symbiosis with host plants, AMF regulates associated gene expression in many secondary metabolic pathways, and then affects the production of secondary metabolites in host plants [45]. P addition up-regulated the expressions of *PcRS*, *PcRS11*, and *PcCRS1* in uninoculated plants and *PcRS11* and *PcCRS1* in inoculated plants, accompanied by the inhibition of *PcRS* expression in inoculated plants (Figure 7), indicating that P addition was dependent on mycorrhizal colonization for the expression of key genes in resveratrol synthesis pathways. Under Ap conditions, AMF colonization up-regulated the expression of *PcSTS*, *PcRS11*, and *PcCRS1*, while under Lp conditions, the expression of *PcRS* and *PcCRS1* was up-regulated by AMF (Figure 7), indicating the importance of this gene in the increase in resveratrol levels by mycorrhizal fungi. AMF promotes the synthesis of terpenoids by promoting the efficiency of P uptake by plants from the soil, which in turn promotes the synthesis of pyrophosphate compounds (isopentene pyrophosphate and dimethyl allyl pyrophosphate) and finally the synthesis of terpenoids [33,46].

5. Conclusions

G. mosseae inoculation dramatically improved plant growth, leaf gas exchange, and P levels of *P. cuspidatum*, dependent on the level of substrate P. To our knowledge, this work revealed for the first time that *P. cuspidatum* plants were more dependent on AMF than P fertilizer for promoting the concentration of active medicinal components in leaves. The improved magnitude by AMF colonization on medicinal components was higher under P-deficient conditions than under P-sufficient conditions. AMF-modulated increases in active medicinal components were associated with expressions of associated genes, especially *PcCRS1*. How mycorrhiza activates the expression of *PcCRS1* in this process is unclear and remains to be studied. Transcriptome sequencing can be used to further screen relevant differentially expressed genes and determine whether this gene expression is specifically induced by AMF and the expression characteristics in arbuscule-contained root cortex cells. It is necessary to pay more attention to the input of suitable P fertilizer and increase the number of AMF populations and the degree of root colonization in *P. cuspidatum*.

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