Arbuscular Mycorrhiza Alters The Nutritional Requirements In *Salvia Miltiorrhiza* And Low Nitrogen Enhances The Mycorrhizal Efficiency

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Research Article

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Abstract

Salvia miltiorrhiza Bunge (*danshen* in Chinese) is one of the most important medicinal cash crops in China. Previously, we showed that arbuscular mycorrhizal fungi (AMF) can promote *S. miltiorrhiza* growth and the accumulation of bioactive compounds. Fertilization may affect mycorrhizal efficiency, and appropriate doses of phosphate (P) and nitrogen (N) fertilizers are key factors for obtaining mycorrhizal benefits. However, the optimal fertilization amount for mycorrhizal *S. miltiorrhiza* remains unclear. In this study, we studied the effects of AMF on the growth and bioactive compounds of *S. miltiorrhiza* under different doses (low, medium, and high) of P and N fertilizer. The results showed that the mycorrhizal growth response (MGR) and mycorrhizal response of bioactive compounds (MBC) decreased gradually with increasing P addition. Application of a low (N25) dose of N fertilizer significantly increased the MGR of mycorrhizal *S. miltiorrhiza*, and a medium (N50) dose of N fertilizer significantly increased the MBC of phenolic acids, but decreased the MBC of tanshinones in mycorrhizal *S. miltiorrhiza*. Our results also showed that the existence of arbuscular mycorrhiza changes nutrient requirement pattern of *S. miltiorrhiza*. P is the limiting nutrient of non-mycorrhizal plants whereas N is the limiting nutrient of mycorrhizal plants.

1 Introduction

Salvia miltiorrhiza Bunge (*danshen* in Chinese) is one of the most important medicinal cash crops in China and is used for treating cerebrovascular and cardiovascular diseases [1], hypertension [2], ischemic stroke [3], breast cancer [4], and hepatitis [5]. *S. miltiorrhiza* is mostly grown under continuous cropping cultivation, which has resulted in serious soil-borne diseases. The high incidence of soil-borne diseases has resulted in the decline of *S. miltiorrhiza* yield and quality, leading to substantial agricultural and economic losses [6].

Arbuscular mycorrhizae are one of the most important symbiotic relationships in terrestrial ecosystems. Arbuscular mycorrhizal fungi (AMF) form a symbiosis with the roots of more than 90% of terrestrial plants, presenting great nutritional and ecological importance [7]. Many studies have reported that AMF can promote plant growth, nutrition uptake (especially phosphate), and increase resistance to biotic and abiotic stresses [8–12]. AMF can also increase the contents of bioactive compounds such as terpenoids, alkaloids, and phenolics in many economically and industrially significant crops and herbs [13–15]. Our previous study also showed that inoculation with *Glomus mosseae* improves growth and salvianolic acid B accumulation in continuously cropped *S. miltiorrhiza* [14]. However, mycorrhizal efficiency varies with soil fertilizers. The content of phosphate (P) and nitrogen (N) in soil significantly affects the interaction between mycorrhiza and plants. In fact, some negative outcomes have also been recorded. For example, P and N fertilization may alter the dynamics of relationships between AMF and plant roots, to reduce the mutualism and even parasitism of AMF [16, 17]. Many studies have shown that varying levels of P and N can have different impacts on the benefits that a plant might receive by altering the relationship dynamics of AMF and plants [18, 19], and that mycorrhizal benefits mainly occur under lower P levels [20].

Rational fertilization is conducive to maximize mycorrhizal benefits. P and N fertilization may cause mycorrhizal effects to turn from positive to negative; however, the optimal fertilization amount for mycorrhizal *S. miltiorrhiza* remains unknown. Therefore, in this study, the effects of different P and N application rates (low, medium, and high) on improving the mycorrhizal efficiency in *S. miltiorrhiza* were examined. We determined the effects of different levels of P and N fertilizer treatment on the percentage of root length colonized (RLC), biomass of shoots and roots of *S. miltirrhiza*, and the contents of phenolic acids (rosmarinic acid, salvianolic acid B), and tanshinones (dihydrotanshinone, cryptotanshinone, tanshinone I, tanshinone IIA) in *S. miltiorrhiza* root. We also evaluated mycorrhizal efficiency based on the mycorrhizal growth response (MGR) and mycorrhizal response of bioactive compounds (MBC) in the root.

2 Results

2.1 Mycorrhizal colonization was decreased by high levels of P fertilizer but increased by N fertilizer

The percentage of the root length colonized (RLC, %) was used as an index to evaluate root colonization by AMF. The roots of all mycorrhizal plants were highly colonized with AMF, whereas those of non-mycorrhizal plants remained free of AMF colonization. According to the statistical results presented in Fig. 2, AMF colonization was affected by P and N fertilization.

The dosage of P fertilizer significantly affected mycorrhizal colonization. The RLC of the P25 treatment was the highest among all P fertilizer treatments, up to 99.19%, and was significantly higher than that of the P0 treatment (Fig. 2a). In contrast, the RLC of the P100 treatment decreased to 80.79%, which was significantly different from other treatments. This indicates that excessive phosphorus fertilizer is not conducive to establish symbiosis between AMF and plants.

In contrast, N fertilizer application increased mycorrhizal colonization. Applying N fertilizer increased the RLC from 91.52–99.04% (Fig. 2b). The N25, N50 and N100 treatments all significantly increased the RLC compared with that of the N0 treatment, and there was no significant difference among the three treatments.

2.2 Application of P reduced the mycorrhizal benefit to plant growth

Applying P fertilizer significantly improved *S. miltiorrhiza* growth under non-mycorrhizal treatments (Fig. 3a). This increase was enhanced by the level of P addition. Compared to the P0 treatment, the P100 treatment significantly increased the fresh weight of shoots by 161%, and that of roots by 155%.

Regardless of P addition, compared with non-mycorrhizal plants, AMF colonization promoted the growth of *S. miltiorrhiza* (Fig. 3a). Under the P0, P25, and P50 treatments, mycorrhizal inoculation significantly increased the biomass of shoots, whereas the P100 treatment did not (Fig. 3a). For roots, the growth

promoting effect can only be achieved without adding P fertilizer (P0) (Fig. 3a). For mycorrhizal plants, the P fertilizer addition significantly inhibited the growth of *S. miltiorrhiza*.

The MGR is correlated to the level of P addition, and decreases as P level increases (Fig. 3b). The MGR under P0 treatment was the highest among all P levels, and the MGR under P100 treatment was the lowest. The MGR of shoot biomass decreased in a consistent manner from 379% (P0) to 55% (P100) whereas the MGR of root biomass decreased from 272% (P0) to 12% (P100). The P0 treatment MGR of the whole plant was 15 times higher than that of the P100 treatment (Fig. 3b).

2.3 Low level application of N fertilizer increased the mycorrhizal benefits to plant growth

Under non-mycorrhizal treatments, the fresh weight of the shoot or root did not increase with the N addition dose (Fig. 3c). Regardless of the N addition dose, compared with non-mycorrhizal plants, AMF colonization significantly increased the biomass of *S. miltiorrhiza* shoots and roots. Under mycorrhizal treatments, the fresh weight of shoots increased gradually with increasing N application. However, the fresh weight of roots under N25 was the highest, and a subsequent increase in nitrogen addition decreased the root biomass. Application of a low level of N (N25) resulted in increased biomass of the shoots by 29-fold and that of the roots by 9.4-fold compared with that in non-mycorrhizal plants (Fig. 3c).

Differences in mycorrhizal efficiency were observed at different N levels. Under the N25 treatment, the MGR of both the shoots and roots was the highest, whereas that of the N0 treatment was the lowest (Fig. 3d). Application of a low level of nitrogen fertilizer (N25) is thus conducive to the development of mycorrhizal benefits to plant growth.

2.4 Presence of phosphate fertilizer was unfavourable for the accumulation of bioactive compounds

The bioactive compounds in the roots of *S. miltiorrhiza* include phenolic acids (rosmarinic acid, salvianolic acid B) and tanshinones (dihydrotanshinone, cryptotanshinone, tanshinone I, tanshinone IIA) (Fig. 1). P fertilizer affected the MBC in the roots of *S. miltiorrhiza*. Under P0 treatment, compared with non-mycorrhizal plants, AMF colonization significantly increased the content of rosmarinic acid by 43.9% (Fig. 4a), salvianolic acid B by 50.9% (Fig. 4b), cryptotanshinone by 134.0% (Fig. 4d), tanshinone IIA by 117.4% (Fig. 4f). AMF colonization also increased the content of dihydrotanshinone (Fig. 4c) and tanshinone I (Fig. 4e), but there was no significant difference. Under the P100 treatment, AMF colonization significantly decreased the content of rosmarinic acid by 34.3%. Under the P25, P50, and P100 treatments, the contents of salvianolic acid B, dihydrotanshinone, cryptotanshinone, tanshinone I, and tanshinone IIA in the roots of the mycorrhizal plant increased or decreased to different degrees, but there was no significant difference.

However, from the perspective of accumulation, mycorrhizal colonization almost increased the accumulation of all bioactive compounds (Fig. 4g). The MBC represents the mycorrhizal response of

bioactive compounds, wherein positive values indicate that mycorrhizal colonization could increase the accumulation of bioactive compounds, whereas negative values indicate that mycorrhizal colonization could decrease the accumulation of bioactive compounds. Under the P0, P25, and P50 treatments, mycorrhizal colonization increased the accumulation of all bioactive compounds (Fig. 4g). Under the P100 treatment, mycorrhizal colonization increased the accumulation of dihydrotanshinone, tanshinone I, and tanshinone IIA, but decreased the accumulation of rosmarinic acid, salvianolic acid B, and cryptotanshinone (Fig. 4g). Under the P0 treatment, the MBC of all six bioactive compounds was the highest. The MBC decreased with the level of P addition (Fig. 4g). This indicates that the absence of phosphorus fertilization increased the MBC and that excessive phosphorus addition decreased the MBC.

2.5 Application of N fertilizer increased the MBC of phenolic acids and decreased the MBC of tanshinones

Without N fertilizer application (the N0 treatment), the content of rosmarinic acid (Fig. 5a), salvianolic acid B (Fig. 5b), cryptotanshinone (Fig. 5d), and tanshinone IIA (Fig. 5f) in mycorrhizal plants was 1.44, 1.51, 2.34, and 2.17 times that in non-mycorrhizal plants. When a high level of N fertilizer was applied (the N100 treatment), mycorrhizal colonization significantly decreased the content of liposoluble constituents such as dihydrotanshinone by 58% (Fig. 5c), cryptotanshinone by 70.0% (Fig. 5d), tanshinone I by 77.7% (Fig. 5e), and tanshinone IIA by 67.55% (Fig. 5f). Overall, AMF increased the content of bioactive compounds in *S. miltiorrhiza* without N fertilizer, but the contrasting result was observed when a high dose of N fertilizer was applied.

Regardless of the N addition dose, mycorrhizal colonization significantly increased the accumulation of bioactive compounds, and the MBC showed all positive values (Fig. 5g). For phenolic acids, a medium dose of N fertilizer application increased the MBC. In contrast, application of N fertilizer decreased the MBC of tanshinones (Fig. 5g).

3 Discussion

Why does a high level of P fertilizer repress AMF colonization and mycorrhizal efficiency? Many reports have shown that P supply represses AMF colonization and mycorrhizal efficiency [21–23], which is consistent with our findings. The MGR and the MBC decreased gradually with the increase in P addition. The MGR of P0 treatment was 15 times higher than that of P100 treatment (Fig. 3b). Under the P0 treatment, the MBC of all six bioactive compounds was the highest. The MBC of phenolic acids decreased from 403.69% to -10.49%, whereas the MBC of tanshinones decreased from 873.48–36.06% (Fig. 4g).

Applying P fertilizer decreased the AMF colonization and mycorrhizal efficiency which could be explained by the least-cost modes of acquisition [24]. Plants preferentially use the cheaper uptake pathway to acquire P. Smith and Smith [25] elucidated two P uptake pathways in arbuscular mycorrhiza-compatible plants: a symbiotic uptake pathway provided by AMF, and a direct uptake pathway through the epidermis and root

hairs [25]. In lower-P soil conditions, non-symbiotic plants need to produce more new fine roots to encounter a given amount of P, whereas symbiotic plants can enhance P uptake primarily by accessing P beyond the root P-depletion zones via the AMF hyphae [26]. The cost of the P uptake pathway via the roots is higher than that via the arbuscular mycorrhizae in lower-P soil conditions. Therefore, the plants showed increased colonization of AMF in lower-P soil conditions. However, in high-P soil conditions, plants do not need to produce more new fine roots and take up P via arbuscular mycorrhizae, and the plant provides 2.3–20% of photosynthetically fixed organic carbon to AMF [27]. Due to a lower cost, in soil conditions with high P levels, plants are likely to favor direct P uptake via the roots rather than uptake via AMF [26]. Therefore, mycorrhizal colonization is consequently inhibited by high levels of P. This is a negative feedback mechanism wherein the plant saves energy due to an optimal supply of nutrients without the fungal symbiont [28]. P fertilizer applied to potted plants leads to the suppression of C flow from the plants to AMF and results in a reduced abundance of AMF in the roots and a decreased relative C income per unit of the AMF biomass [27].

Applying N fertilizer increases mycorrhizal colonization and MGR. AMF colonization is influenced by the available nutrient contents in soil [29, 30]. Our results showed that applying a low level of N fertilizer significantly increased AMF colonization and the MGR. Application of a low level of N (N25) resulted in increased biomass of the shoots by 29-fold and that of the roots by 9.4-fold compared with that in non-mycorrhizal plants (Fig. 3c). This is in accordance with previous studies [19, 31]. Wu et al. showed that N fertilizer increased AMF colonization regardless of the N addition dose [19]. Püschel et al. also reported that the application of low and medium N levels increased the mycorrhizal efficiency [31].

Applying N fertilizer significantly increased AMF colonization and the MGR because N fertilizer is required for both plant and fungal growth. A limited supply of N in soil could create competition for N, therefore potentially reducing the net mycorrhizal benefits [18]. N application can improve the N supply in soil, thus eliminating the competition between mycorrhiza and plants, and significantly improving mycorrhizal colonization and mycorrhizal efficiency. We thus suggest that 25 mg•kg^{-1} N fertilizer should be maintained in soil during the mycorrhizal cultivation of *S. miltiorrhiza*.

The limiting nutrient of non-mycorrhizal plants is P, whereas that of mycorrhizal plants is N. The ‘law of the minimum’ indicates that plant growth may be controlled by a single necessary resource, and the supply of this resource is limited [32]. Our results showed that P or N fertilizer had different effects on the growth and accumulation of bioactive compounds in non-mycorrhizal and mycorrhizal plants. Applying P fertilizer significantly increased the biomass of non-mycorrhizal plants, whereas the contrasting effect was found on on mycorrhizal plants (Fig. 3a). N fertilizer did not significantly affect the biomass of non-mycorrhizal plants, but significantly increased the biomass of mycorrhizal plants (Fig. 3c). Our results showed that P was the limiting nutrient for non-mycorrhizal plants, whereas N was the limiting nutrient for mycorrhizal plants. Inoculation of AMF can alter the nutrient demand of mycorrhizal plants. Reports have shown that AMF acquire a large amount of N from soil for their growth and can help plants take up P, resulting in N deficiency and P sufficiency for mycorrhizal plants [10, 18].

The MBC of tanshinones and phenolic acids had different responses to N fertilizer supply. N or P fertilizer supply to plants has been shown to have varying effects on the production of secondary metabolites according to the compounds and plant species [33]. N fertilizer reduced the phenolic compounds of Moringa oleifera [34], increased the monoterpenoids content of Thuja plicata [35], and did not significantly affect the anthocyanin concentrations in the berries of Vaccinium myrtillus [36]. Our results showed that when a medium dose of N fertilizer was applied (N50), the MBC of phenolic acids was 2.37 times that observed in the N0 treatment; however, the MBC of tanshinones decreased to 50% of that with the N0 treatment (Fig. 5g). The MBC decrease of tanshinones is consistent with the carbon-nutrient balance hypothesis (CNBH). The CNBH presumes that increasing availability of nutrients reduces the concentration of carbon-based secondary metabolites [37]. The MBC increase of phenolic acids is in contrast to that observed for CNBH, because the biosynthesis of salvianolic acid B and rosmarinic acid occurs via the shikimate/phenylpropanoid pathway. Phenylalanine and tyrosine are precursors of salvianolic acid B and rosmarinic acid [38]. N is essential for amino acid synthesis. In this study, N application could promote the synthesis of phenylalanine and tyrosine, leading to the accumulation of salvianolic acid B and rosmarinic acid in mycorrhizal S. miltiorrhiza.

4 Conclusion

S. miltiorrhiza cultivation requires both AMF inoculation and fertilization to achieve optimal growth and yields; further, AMF application can reduce the amount of chemical fertilizers, offering a more sustainable farming system that is environment-friendly. P and N fertilizers affected AMF colonization and mycorrhizal efficiency in *S. miltiorrhiza*. P fertilizer was not conducive to mycorrhizal benefits as the MGR and MBC gradually decreased with increasing P addition. Application of low-dose N fertilizer (N25) significantly increased the MGR, whereas medium-dose N fertilizer (N50) significantly increased the MBC of phenolic acids, but decreased the MBC of tanshinones. Our result also showed that mycorrhizal and non-mycorrhizal plants have different responses to P or N fertilizer application. We found that the limiting nutrient of non-mycorrhizal plants is P, whereas that of mycorrhizal plants is N. Therefore, we suggest that P fertilizer should not be applied in the mycorrhizal cultivation of *S. miltiorrhiza*, whereas the amount of N fertilizer can be adjusted as required. Considering yield, 25 mg of N fertilizer per kilogram of soil should be applied. However, when targeting the content of bioactive compounds and accumulation of tanshinones, no N fertilizer should be applied. When targeting the accumulation of phenolic acids, 50 mg N fertilizer per kilogram of soil should be applied. Overall, these findings provide information for optimal the cultivation of *S. miltiorrhiza* with specific targets.

5 Methods

5.1 Plant materials, soil, and fungal inoculants

The seeds of *S. miltiorrhiza* used in this study were collected from the Laiwu Danshen cultivation base in Shandong Province, China (36°20’ N, 117°41’ E), and complied with relevant institutional, national, and

international guidelines and legislation. We have obtained the permission to collect seeds.

Soil used for the pot experiment was collected from the top 0—20 cm of soil at the Laiwu Danshen cultivation base. The soil was sieved to < 4 mm and was then sterilized at 121°C for 60 min, for 7 consecutive days before initiating the experiment. Soil from this site was classified as weathered rock (50% rock) with the following values: pH 8.44, organic material 4.94 g•kg^{-1}, total nitrogen 240 mg•kg^{-1}, total phosphorus 380 mg•kg^{-1}, total potassium 18400 mg•kg^{-1}, available nitrogen 35.1 mg•kg^{-1}available phosphorus 2.3 mg•kg^{-1}, and available potassium 28 mg•kg^{-1}.

Mycorrhizal inocula were obtained from the sand cultures of *Glomus versiforme*, which were originally provided by Professor Honggang Wang (Beijing Chinese Academy of Agricultural Sciences, China). White clover (*Trifolium repens*) was used as the host. The inocula contained sand, spores, hyphae, and mycorrhizal roots with an average concentration of 20 spores•g^{-1}.

5.2 Experimental design

The pot experiment with cultivated *S. miltiorrhiza* was conducted in a greenhouse in Beijing City, China (116°43’ E, 39°59’ N). The seeds were collected at the cultivation base mentioned before. To avoid any competing organisms, seeds were first sterilized before sowing as follows: submerging in 75% ethanol for 2 min, then transfer to a 10% H_2O_2 solution for 10 min, washing with tap water for 3 min, and finally soaking in sterile water at room temperature for 12 h. The sterilized seeds were then sown in pots containing moist sterilized vermiculite. Two seedlings were transplanted into each experimental pot after 30 days from the time of sowing; the pots and soil were kept at 25°C during transplantation.

The amount of P and N fertilizer used for the field cultivation of *S. miltiorrhiza* is generally 50 mg KH_2PO_4 and urea per kilogram of soil (150 kg•ha^{-1}); therefore, three fertilization treatments including high, medium, and low doses were designed. Four concentrations of P fertilizer (natural baseline amount and further addition of 25, 50, and 100 mg KH_2PO_4 per kilogram of soil) were applied with or without AMF inoculation as the P0, P25, P50, and P100 treatments, respectively. Four concentrations of N fertilizer (natural baseline amount and further addition of 25, 50, and 100 mg urea per kilogram of soil) were applied with or without AMF inoculation as the N0, N25, N50, and N100 treatments, respectively. Thus, there were 16 treatments in our experiment. As each treatment had 6 replicates, there were 96 pots in the experiment. The AMF treatments (AM) were inoculated with 10 g of inoculum layered below the seeds in each pot, and the non-mycorrhizal treatments (NM) received 10 g of autoclaved inoculum. Plants were watered with distilled water when necessary and incubated at a temperature of 22—35°C. The pots were randomly arranged in the greenhouse. The plants were harvested at 4 months after transplantation, and the fresh weights of the shoots and roots were recorded.

5.3 Estimation of AMF colonization

Fresh fibrous root samples were used to visualize AMF colonization. To determine AMF colonization, the fibrous roots of *S. miltiorrhiza* were cut into small pieces (approximately 1 cm long). The roots were then

stained with Trypan Blue following the procedure proposed by Phillips and Hayman [39]. AMF colonization was determined using the method described by Giovannetti and Mosse [40].

5.4 Assessment of plant growth

After harvesting, the above and below-ground portions were separated, and the fresh weights of both the aerial portion and roots were recorded.

5.5 Quantification of bioactive compounds in roots

The contents of rosmarinic acid (Fig. 1a), salvianolic acid B (Fig. 1b), dihydrotanshinone (Fig. 1c), cryptotanshinone (Fig. 1d), tanshinone I (Fig. 1e), and tanshinone IIA (Fig. 1f) in the roots were determined using the methods described by Chen et al. (2017b). Roots harvested from experimental plants were dried at 40°C for 48 h. The dried roots were ground in a standard grinder and then passed through a 40-mesh sieve. Then, 10 ml of methanol: water solution (80:20) was used to extract the sieved root (0.1 g) in an ultrasonic bath for 30 min at room temperature. After extraction, the solution was filtered through a 0.45 μm filter, and the filtrate was collected. A 10 μL aliquot of the filtrate was injected, and separated using HPLC with a C18 Symmetry® column (4.0 mm × 250 mm, 3 μm; Waters Corp., Milford, MA, United States). The mobile phase A comprised a water-phosphoric acid solution (A; 100:0.10, v/v), and the mobile phase B was acetonitrile. The gradient elution program was as follows: (0–15) min, 75% B; (15–16) min, 25%→40% B; (16–18) min, 40%→50% B; (18.0–55.0) min, 50%→70% B; (55.0–65.0) min, 75%→25% B. Eluted compounds were detected spectrophotometrically at 280 nm using a 996 PDA photodiode array detector (Waters Corp., Milford, MA, United States). The column temperature was set at 25°C; the flow rate was 1.0 $mL \bullet min^{-1}$.

5.6 Statistical analysis

The parameters of the MGR and MBC were calculated as $(AM\text{-}NM_{mean})/NM_{mean} \bullet 100\%$ (Voříšková et al., 2019), where AM is the value (shoot biomass, root biomass, or accumulation of bioactive compounds in the roots of *S. miltiorrhiza*) of inoculated plants in the AM treaments and NM_{mean} is the mean value of non-inoculated plants in the corresponding NM treatments. All data were analyzed using IBM SPSS Statistics 24. Results were presented as the mean ± SD of six replicates. The data of the RLC, fresh weight of shoots and roots, MGR, and MBC were subjected to one-way ANOVA followed by the least significant difference (LSD) test. The data of the contents of bioactive compounds were subjected to the t-test. Differences were reported as significant when $P < 0.05$.

Declarations

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Chunjuan Pu], [Pengying Li], [Xiuteng Zhou] and [Guang Yang]. The first draft

of the manuscript was written by [Chunjuan Pu] and [Meilan Chen] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing Interests: The authors declare that they have no conflict of interest.

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Figures

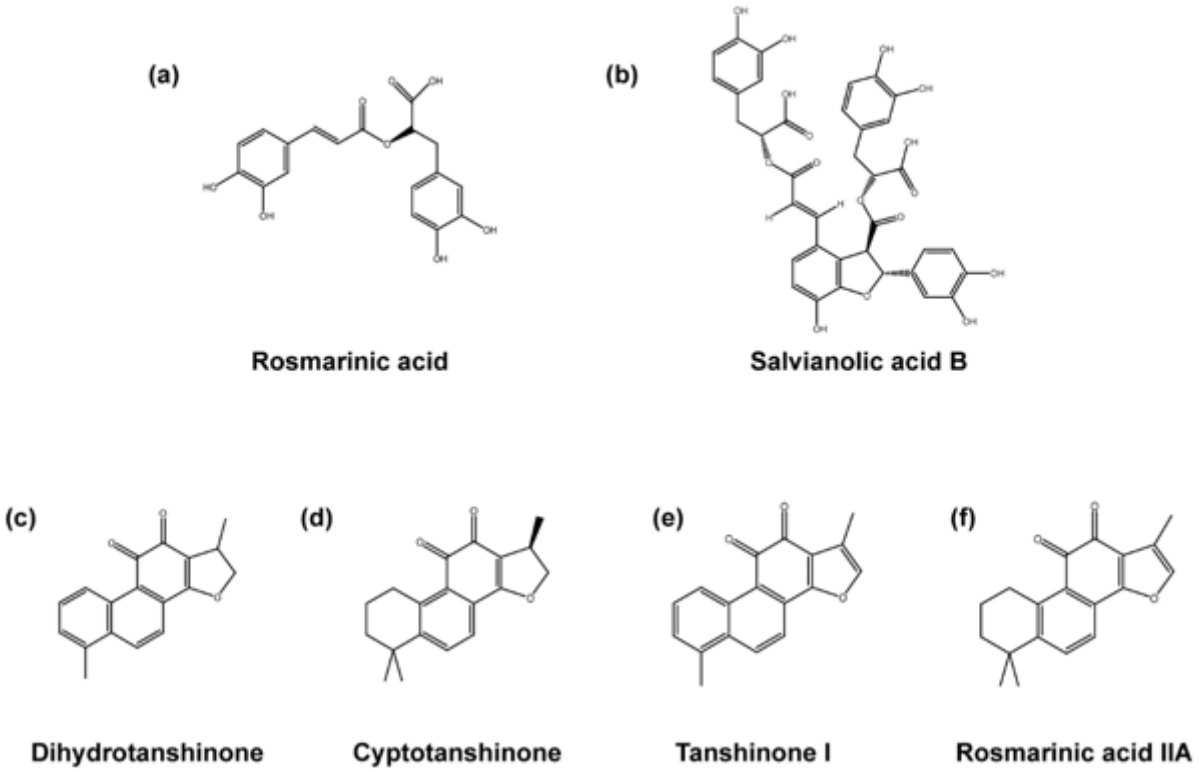

Figure 1

Bioactive compounds detected in *S. miltiorrhiza* and their chemical structures. (a) and (b) are the phenolic acids (rosmarinic acid, salvianolic acid B). (c-f) are the tanshinones (dihydrotanshinone, cryptotanshinone, tanshinone I, and tanshinone IIA)

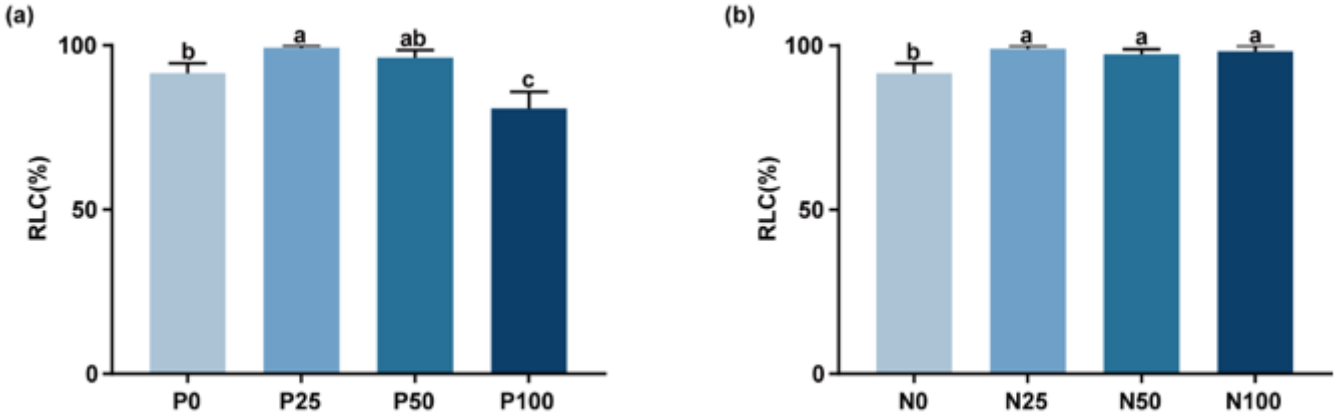

Figure 2

Mycorrhizal colonization of *S. miltiorrhiza* inoculated with *G. versiforme* affected by the addition of P (a) and N (b) fertilizer. (a) The P0, P25, P50, and P100 treaments represent the four levels of P fertilizer

application (0, 25, 50, and 100 mg P added per kilogram of soil, respectively). (b) The N0, N25, N50, and N100 treatments represent the four levels of N fertilizer application (0, 25, 50, and 100 mg N added per kilogram of soil, respectively). Percentage of root length colonized (RLC, %) is expressed as the percentage of root length occupied by mycorrhizal fungal structures such as hyphae, arbuscules, and/or vesicles. Means ± standard errors are shown (n = 6). Different lowercase letters indicate significant differences between different treatments according to one-way ANOVA followed by the least significant difference test ($p < 0.05$). The roots of control non-mycorrhizal plants remained free of mycorrhizal fungal structures (data not shown)

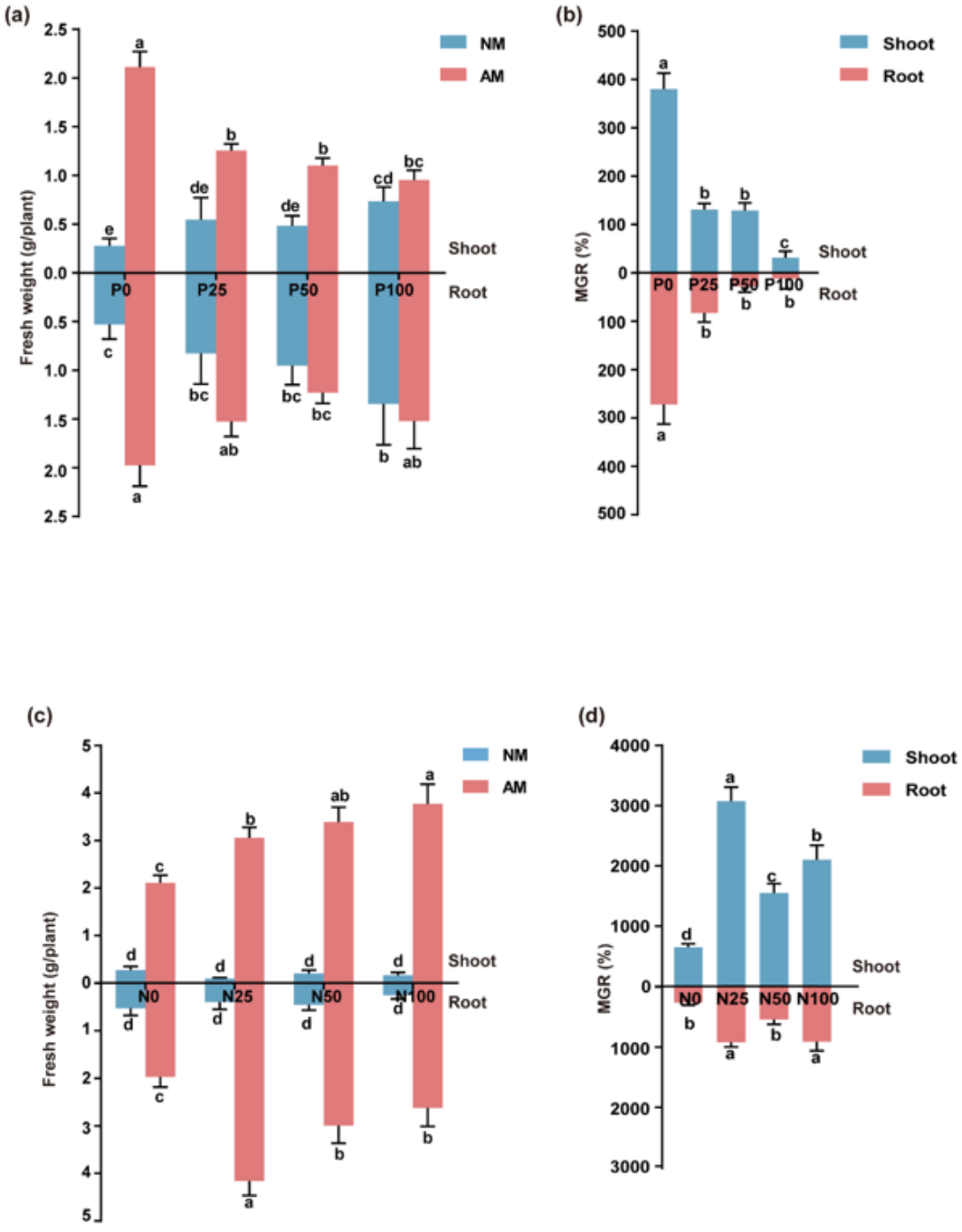

Figure 3

Mycorrhizal efficiency on plant growth under different levels of P (0, 25, 50, and 100 mg P added per kilogram of soil; P0, P25, P50, P100) and N (0, 25, 50, and 100 mg N added per kilogram of soil; N0, N25, N50, N100) fertilizer. (a) and (c) show the fresh weight of *S. miltiorrhiza* shoots and roots under different treatments. The mycorrhizal growth response (MGR) of plants along a P (b) or N (d) fertilization gradient consisting of four input levels. The means ± standard errors are shown (n = 6). Different lowercase letters

indicate significant differences between different treatments according to one-way ANOVA followed by the least significant difference test ($p < 0.05$)

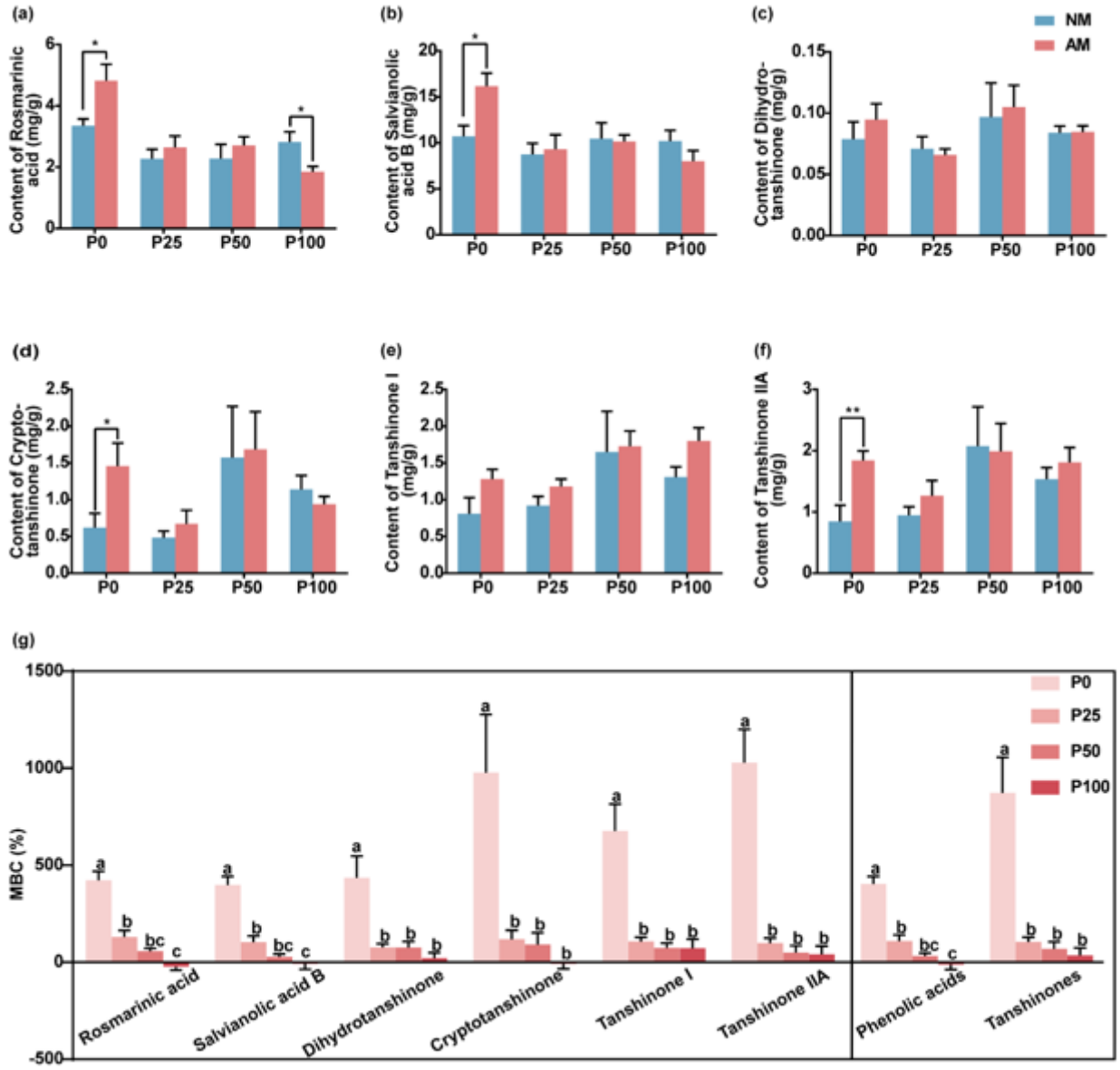

Figure 4

Mycorrhizal response of bioactive compounds (MBC) under different levels of P (0, 25, 50, and 100 mg P added per kilogram of soil; P0, P25, P50, P100) fertilizer. (a-f) The content of bioactive compounds, rosmarinic acid (a), salvianolic acid (b), dihydrotanshinone (c), cryptotanshinone (d), tanshinone I (e), and tanshinone IIA (f) in the roots of *S. miltiorrhiza* under different treatments. The means ± standard errors are shown (n = 6). Asterisks indicate significant differences between arbuscular mycorrhizal plants and non-mycorrhizal plants according to the t-test (*$0.01 \leq p \leq 0.05$, **$0.001 \leq p \leq 0.01$, *** $p \leq 0.001$). (g) Mycorrhizal response of bioactive compounds (MBC) under different levels of P. The means ± standard errors are shown (n = 6). Different lowercase letters indicate significant differences between different treatments according to one-way ANOVA followed by the least significant difference test ($p < 0.05$)

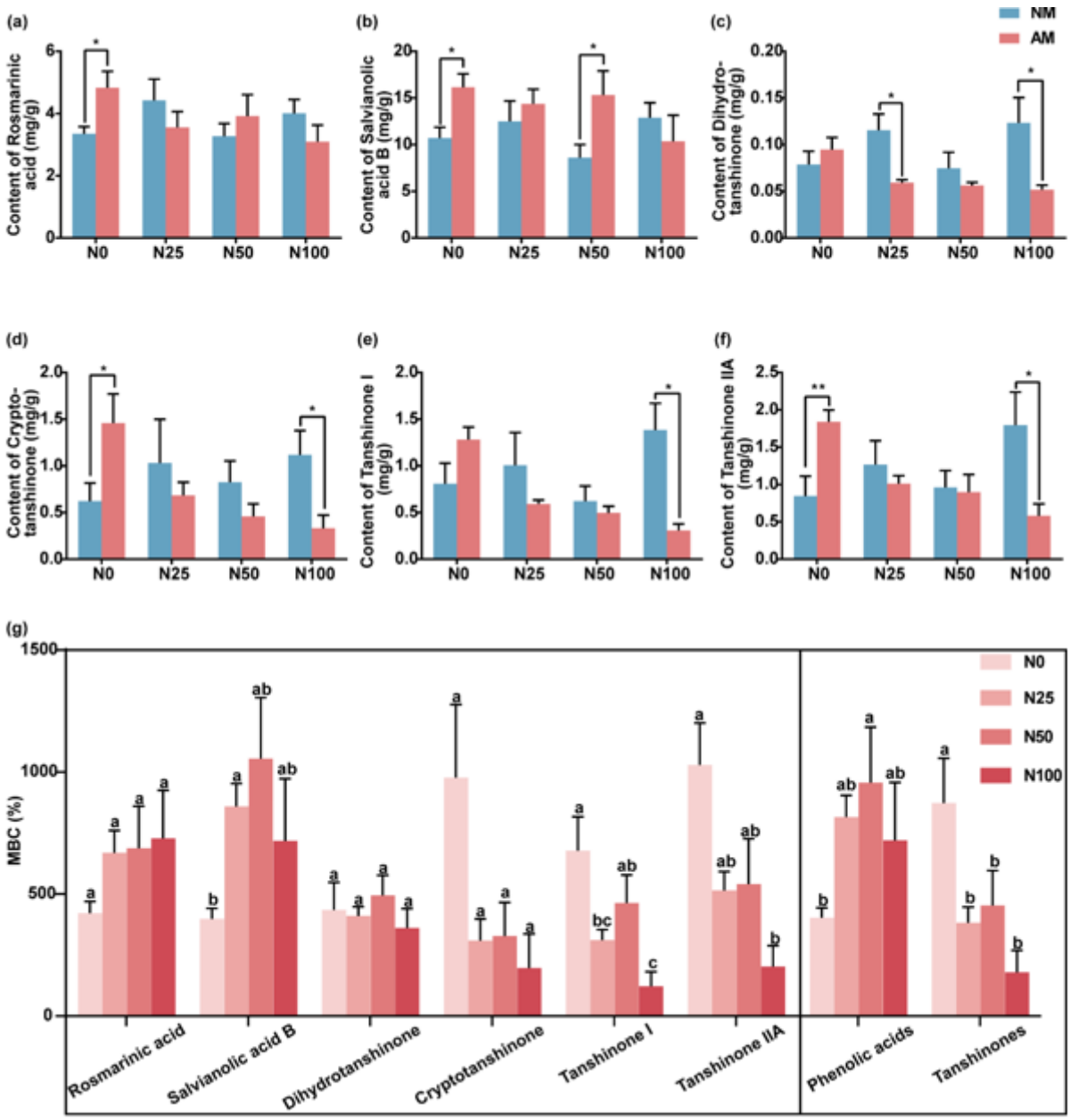

Figure 5

Mycorrhizal response of bioactive compounds (MBC) under different levels of N (0, 25, 50, and 100 mg N added per kilogram of soil; N0, N25, N50, N100) fertilizer. (a-f) The content of bioactive compounds, rosmarinic acid (a), salvianolic acid (b), dihydrotanshinone (c), cryptotanshinone (d), tanshinone I (e), tanshinone IIA (f) in the roots of *S. miltiorrhiza* under different treaments. The means ± standard errors are shown (n = 6). Asterisks indicate significant differences between arbuscular mycorrhizal plants and non-mycorrhizal plants according to the t-test (*$0.01 \leq p \leq 0.05$, **$0.001 \leq p \leq 0.01$, *** $p \leq 0.001$). (g) Mycorrhizal response of bioactive compounds (MBC) under different levels of N. The means ± standard errors are shown (n = 6). Different lowercase letters indicate significant differences between different treatments according to one-way ANOVA followed by the least significant difference test ($p < 0.05$)