Effects of arbuscular mycorrhizal fungi on the adaptability of Sophora davidii under low phosphorus stress

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Research Article

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Abstract

The lack of phosphorus in the soil is one of the important factors restricting the healthy growth of *Sophora davidii* in karst areas, and improving the absorption of phosphorus is of great significance to the growth and utilization of *S. davidii*. Arbuscular mycorrhizal fungi (AMF) can form a mutualistic symbiotic relationship with plants, which can relieve stress and promote the growth of host plants. A sand culture method was used in this study. *S. davidii* seedlings inoculated with AMF and without AMF (NAM) were used as research materials under three phosphatase treatments, P0.5 (0.5 mmol/L), P0.25 (0.25 mmol/L), and P0 (0 mmol/L), to investigate the effects of arbuscular mycorrhizal fungi on the growth, morphology, physiology, endogenous hormone contents and accumulation of N and P elements in *S. davidii* seedlings. The results showed that under NAM, the plant height, growth rate, aboveground dry weight and chlorophyll content were significantly decreased under low phosphorus stress. Under low phosphorus stress (P0.25) intensity, *S. davidii* showed increased root dry weight, root shoot ratio, total root length, root surface area, root tip number, and root hair number, an increased content of osmotic adjustment substances such as proline, soluble sugar and soluble protein, increased activity of protective enzymes such as acid phosphatase and catalase, and increased hormone contents to adapt to the low phosphorus stress. However, when the low phosphorus stress intensity increased to P0, the regulatory effect was severely weakened. Inoculation with AMF promoted the growth of the aboveground parts of *S. davidii*; although it decreased the growth of the root system and root biomass, it significantly increased the total root length, root tip number and growth rate under P0 stress and increased chlorophyll a, total chlorophyll, proline, soluble sugar, soluble protein, and IAA contents, and acid phosphatase and superoxide dismutase activities. The roots of *S. davidii* and AMF engage in symbiosis, improving root morphology, promoting the absorption of nitrogen and phosphorus by seedlings, and maintaining the N:P balance of leaves, thereby maintaining a higher biomass of *S. davidii* under low phosphorus stress and relieving the low phosphorus stress on seedlings. Our results demonstrated that AMF inoculation is useful for the promotion and cultivation of *S. davidii* in the karst area of Southwest China under low phosphorus stress conditions. Therefore, in the process of ecological restoration and forage improvement using *S. davidii* on acidic soil in the karst region of Southwest China, inoculation with AMF may be a good strategy to stabilize *S. davidii* yields.

Introduction

Phosphorus is a necessary macroelement for plant growth and development; it is a component of important compounds such as phospholipids, nucleic acids, and nucleoproteins and participates in physiological and biochemical processes such as plant metabolism, photosynthesis, and respiration[1-2]. According to statistics, more than 5.7 billion hectares of arable soil worldwide are deficient in available phosphorus[3]. Plants absorb phosphorus in the form of acid phosphates ($H_2PO_4^-$, HPO_4^{2-})[4]. Acid phosphates are present in soil solutions at concentrations of 0.1-10 μM L^{-1}[5]. The application of phosphorus fertilizer can alleviate the shortage of soil available phosphorus to a certain extent; however, phosphorus has low mobility in soil and is easily fixed. Phosphate is easily formed with soil particles or metal ions (Ca^{2+}, Fe^{2+}, etc.), which reduces the absorption and utilization of soil phosphorus by plants[6-7]. Studies have reported that a long-term low-phosphorus environment reduces plant nodule development and nitrogen fixation ability and increases flower shedding and plant dwarfism. The plant seedling emergence rate is low, and seed size and yield are rapidly reduced[8]. The decrease in plant growth and productivity caused by low phosphorus stress is the main challenge facing current production practices. Therefore, how to increase the available phosphorus content in the soil and improve phosphorus use efficiency through biological measures is a key problem to be solved in current forage production.

Rhizosphere microorganisms dissolve and mineralize poorly soluble organic and inorganic phosphates in soil[9-10]; thus, they can improve the absorption and utilization of phosphorus by crops. In particular, arbuscular mycorrhizal fungi (AMF) can form a mutualistic relationship with 80% of terrestrial plants[11]. Plants deliver carbohydrates to mycorrhizal fungi, which deliver nutrients and water to plants to improve host plant resistance to biotic and abiotic stresses[12-13]. Various plant studies have shown that arbuscular mycorrhiza (AM) formation increases antioxidant enzyme activity[14], reduces the accumulation of malondialdehyde and hydrogen peroxide[14], and enhances the accumulation of soluble sugars in host plants[15]. Increases in host plant biomass and leaf photosynthesis promote phosphorus absorption and utilization[16]. It has also been reported that AMF hyphae can produce gibberellins (GA) and cytokinins (CTKs) and can also synthesize auxin (indole-3-acetic acid, IAA), gibberellin (GA_3) and ethylene, which play an important role in the symbiosis between AMF and plants, being beneficial for plant adaptation to low phosphorus environments[17]. Therefore, exploring the effects and mechanisms of mycorrhizae on plant growth and physiology is of great significance for improving phosphorus utilization efficiency, coping with adversity, and promoting agricultural development.

Sophora davidii is a perennial deciduous shrub of the Fabaceae family. *S. davidii* is widely distributed in northwestern and southwestern China, especially in the acidic soils of karst areas in the southwest[18-19]. *S. davidii* has high feed, medicinal and ecological protection value[19]. Because of its high resistance to stress and high nutritional value, it is widely used in ecological restoration and for feed improvement[20]. However, the acidic soil in the south exhibits strong phosphorus fixation and low availability of soil nutrients (N, P)[19]. Thus, the growth and productivity of *S. davidii* is harmed by these low phosphorus conditions. At present, research on *S. davidii* has mainly focused on low phosphorus stress[21], drought stress[22] and so on. However, there are few reports on the use of AMF to explore the phenotypic adaptations and physiological responses of *S. davidii*. To further improve the stress resistance of *S. davidii* and accelerate the utilization of *S. davidii* germplasm in karst mountainous areas, the sand culture method was used in this experiment to explore the effects of AMF on the low phosphorus tolerance of *S. davidii*. These findings provide a theoretical basis for using AMF to fully promote and utilize *S. davidii* resources.

1 Materials And Methods

1.1 Experimental materials

The plant material used was *S. davidii* preserved in the laboratory; the arbuscular mycorrhizal fungus was *Funneliformis mosseae*, purchased from the College of Horticulture, Yangtze University.

1.2 Experimental design

Plump and uniform seeds of *S. davidii* were soaked and germinated. At 14 days after germination, seedlings with uniform growth were transplanted into 4.5 L plastic pots with quartz sand and vermiculite (1:1), with 10 plants in each pot. The roots were inoculated with 10 g of AMF-containing culture soil (AM) or high-temperature sterilized AMF culture soil (NAM), and plants were allowed to grow for 60 days. During the experimental growth period, the temperature in the greenhouse was controlled at 20-25°C, and the humidity was 60%. During this period, Hoagland nutrient solution was added every two days to ensure that the plant nutrient and water requirements were met. Phosphorus stress started after 60 days, and 3 phosphorus treatments were set for the AMF and NAM treatment groups: P0.5 (0.5 mmol/L KH_2PO_4 Hoagland nutrient solution), P0.25 (0.25 mmol/L KH_2PO_4 Hoagland nutrient solution), and P0 (0 mmol/L KH_2PO_4 Hoagland nutrient solution). HCl and NaOH were used to adjust the pH value of the nutrient solution to 6.0±0.1, and KH_2PO_4 was used as a phosphorus source. Samples were taken at 14 days of phosphorus stress treatment and stored at -80°C.

The composition of the Hoagland nutrient solution was as follows: 1 mmol/L KNO_3, 2 mmol/L $Ca(NO_3)_2$, 1 μmol/L H_3BO_3, 1.5 mmol/L $CaCl_2$, 1.8 mmol/L KCl, 0.5 mmol/L $MgSO_4$, 1 μmol/L MnSO4, 0.5 μmol/L CuSO4, 1 μmol/L ZnSO4, 0.1 μmol/L $(NH_4)_6Mo_7O_{24}$, 0.05 μmol/L $FeSO_4$, and 50 μmol/L Na_2-EDTA[23].

1.3 Index determination methods

1.3.1 Measurement of AMF colonization

Seedling roots were first washed with running tap water and then several times with distilled water. Samples were randomly cut into 1-cm-long root fragments. The roots were cleared for 15 min in 10% (w/v) KOH at 90°C, bleached in alkaline hydrogen peroxide for 20 min, acidified in 1% (v/v) HCl, and stained in trypan blue at 90°C for 30 min. Samples were then decolorized with a mixture of lactic acid and glycerin (v/v = 1:1) three times[24]. The AMF colonization rate was calculated according to the magnifying cross method[25]. The colonization rate of AMF was determined as follows: AMF colonization rate= (hyphae intersection+vesicle intersection+arbuscular intersection)/total intersection×100%.

1.3.2 Measurement of growth indexes

Plant height was measured with a ruler, and each treatment was repeated 5 times. Root and leaf samples were captured with an Epson Perfection V800 photo scanner. The root parameters (root surface area, root volume, total root length, average root diameter and root tip number) were analyzed using Win-RHIZO software (Regent Instructions, Canada Inc.). The leaf parameters (leaf width, leaf length, leaf surface area and leaf perimeter) were analyzed using WinFOLIA software (Regent Instructions, Canada Inc.). The fresh seedlings were divided into aerial parts and roots, placed in an oven at 105°C for 20 minutes, and dried at 65°C to a constant weight followed by weighing. Each treatment was repeated 3 times. The root-shoot ratio was calculated using the formula: root-shoot ratio = dry weight of the roots (mg)/dry weight of the aerial part (mg).

1.3.3 Measurement of physiological indexes

The contents of proline (Pro), soluble sugar (SS), soluble protein (SP), malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), chlorophyll and acid phosphatase (ACP) in roots were determined using the respective Solarbio detection kits (Beijing Solarbio Science & Technology Co., Ltd., Beijing).

1.3.4 Measurement of endogenous hormones

Auxin (indole-3-acetic acid, IAA), gibberellin (GA_3), and brassinolide (BR) levels in leaves and roots were determined using high-performance liquid chromatography[26-27].

1.3.5 Measurement of mineral elements

Powdered root, stem, and leaf samples were sieved through a 0.45 mm sieve, and the N and P concentrations were determined using the Kjeldahl method and molybdenum-stibium colorimetry[28], respectively.

1.4 Data processing

Microsoft Excel 2010 was used for data sorting, and Duncan's multirange test method was used to perform variance analysis on the various *S. davidii* parameters (SPSS Inc., Chicago, version 18.0). SigmaPlot 14.0 was used to draw the graphs.

2 Results And Analysis

2.1 Root AMF colonization rate

Significant mycorrhizal colonization was observed in mycorrhizae-inoculated seedlings. Under the conditions of P0.5 (Figure 1B), P0.25 (Figure 1C) and P0 (Figure 1D), mycorrhizal hyphae finally formed different morphological structures, indicating that the AMF could form a symbiotic relationship with the seedlings. Inoculation with AMF significantly increased the AMF colonization rate in the roots of *S. davidii* seedlings ($P < 0.05$) (Figure 1E). The lower the

phosphorus concentration was, the higher the AMF infection rate ($P < 0.05$) of *S. davidii* seedlings, and the AMF infection rates showed the following trend: P0>P0.25>P0.5.

2.2 Effects of AMF on the growth indexes of *S. davidii* under low phosphorus stress

2.2.1 Effects of AMF on the plant height and biomass of *S. davidii* under low phosphorus stress

The effects of AMF on the plant height and biomass of *S. davidii* under low-phosphorus stress are shown in Table 1. With the intensification of phosphorus stress (inoculated or not inoculated with AMF), plant height and aboveground dry weight showed a downward trend, while root dry weight showed an upward trend. With AMF inoculation, the root-shoot ratio increased, and the growth rate decreased with the intensification of phosphorus stress. Phosphorus stress significantly increased the root dry weight and root-shoot ratio and significantly decreased the aboveground dry weight ($P < 0.05$). Compared with NAM, inoculation with AMF significantly increased the growth rate under the P0 treatment, significantly increased the plant height and aboveground dry weight under the P0.25 and P0 treatments, and significantly decreased the root dry weight and root-shoot ratio under the P0.25 and P0 treatments ($P < 0.05$).

Table 1 Effects of AMF on plant height and biomass of *S. davidii* under low-phosphorus stress

P Status	AMF Status	Plant height (mm)	Growth rate (mm/d)	Root dry weight (g)	Aboveground dry weight (g)	Root shoot ratio
P0.5	NAM	192.50±17.23ab	1.07±0.21a	0.08±0.06c	0.53±0.10a	0.14±0.08c
	AM	214.25±15.91a	1.27±0.18a	0.06±0.03c	0.52±0.15a	0.11±0.04c
P0.25	NAM	185.65±10.71bc	0.56±0.17b	0.24±0.05a	0.36±0.09b	0.71±0.30a
	AM	211.30±9.82a	0.63±0.28b	0.12±0.04b	0.50±0.04a	0.34±0.07b
P0	NAM	168.00±8.48c	0.50±0.26b	0.36±0.11a	0.32±0.21b	0.80±0.35a
	AM	193.40±17.66ab	1.13±0.23a	0.15±0.13b	0.50±0.07a	0.30±0.01b
	AMF	**	*	*	*	**
Significance	P	*	**	**	**	**
	AMF×P	NS	*	*	*	*

Note: different lowercase letters indicate significance at $P < 0.05$. Values are means ± SE. NAM, non-AMF-inoculated; AM, AMF-inoculated. AMF×P, interaction between AMF inoculation and phosphorus stress. *$P < 0.05$, **$P < 0.01$. NS, no significant effect. The same applies below.

2.2.2 Effects of AMF on root morphology of *S. davidii* under low-phosphorus stress

The effect of AMF on the root morphology of *S. davidii* under low phosphorus stress is shown in Table 2. Without AMF inoculation, the total root length, total root surface area, root tip number and root hair number increased first and then decreased with increasing phosphorus stress intensity, and all were the largest under the P0.25 treatment, with values significantly larger than those under the other treatments ($P < 0.05$). Compared with NAM, inoculation with AMF significantly reduced the total root length and total root surface area in the P0.25 and P0.5 treatments and significantly decreased the number of root tips and root hairs in the P0.25 treatment. The total root length and root tip number were significantly increased in the P0 treatment ($P < 0.05$). Inoculation with AMF, phosphorus stress and the interaction of AMF×P resulted in no significant difference in root volume and root diameter ($P > 0.05$). After inoculation with AMF, the total root length, total root surface area and root hair number were the largest under the P0 treatment, with values of 125.78 cm, 35.86 cm^2 and 611.67, respectively.

Table 2 Effects of AMF on root morphology of *S. davidii* under low-phosphorus stress

P Status	AMF Status	Total root length (cm)	Total root surface area (cm^2)	Root volume (cm^3)	Root Diameter (mm)	Root tip number	Root hair number
P0.5	NAM	112.45±19.81b	33.54±8.75b	0.81±0.33a	0.95±0.17a	181.00±70.53c	508.33±107.82b
	AM	86.94±10.16d	26.76±5.28c	0.67±0.25a	0.98±0.21a	207.67±75.96bc	494.00±10.54b
P0.25	NAM	206.93±39.72a	51.65±8.63a	1.03±0.15a	0.80±0.02a	327.67±87.32a	972.33±300.27a
	AM	101.31±21.80c	26.62±4.07c	0.57±0.14a	0.85±0.14a	206.00±37.04bc	588.00±118.20b
P0	NAM	97.23±7.52d	33.22±4.24b	0.93±0.27a	1.10±0.20a	146.67±59.43c	561.67±14.34b
	AM	125.78±48.00b	35.86±17.95b	0.82±0.51a	0.89±0.13a	273.67±57.43ab	611.67±338.17b
	AMF	*	*	NS	NS	*	*
Significance	P	*	*	NS	NS	*	*
	AMF × P	**	*	NS	NS	NS	NS

2.3 Effects of AMF on physiological indexes of *S. davidii* under low-phosphorus stress

2.3.1 Effects of AMF on the chlorophyll content of *S. davidii* under low phosphorus stress

The effect of AMF on the chlorophyll content of *S. davidii* under low phosphorus stress is shown in Table 3. Under NAM, chlorophyll a, chlorophyll b, and total chlorophyll contents and the chlorophyll a/b ratio were not significantly different among treatments ($P > 0.05$). Inoculation with AMF and the interaction of AMF×P had significant effects on chlorophyll a and total chlorophyll contents ($P < 0.05$). Compared with NAM, inoculation with AMF significantly increased the chlorophyll a and total chlorophyll contents in the P0.25 and P0 treatments ($P < 0.05$).

Table 3 Effects of AMF on chlorophyll contents in leaves of *S. davidii* under low-phosphorus stress

P Status	AMF Status	Chlorophyll a ($mg·g^{-1}$)	Chlorophyll b ($mg·g^{-1}$)	Chlorophyll a/b ($mg·g^{-1}$)	Total chlorophyll ($mg·g^{-1}$)
P0.5	NAM	0.81±0.21ab	0.38±0.09a	2.14±0.11a	1.19±0.30ab
	AM	0.67±0.33b	0.33±0.10a	1.97±0.45a	1.00±0.43b
P0.25	NAM	0.65±0.19b	0.30±0.06a	2.12±0.20a	0.95±0.25b
	AM	0.92±0.16a	0.43±0.10a	2.17±0.37a	1.35±0.23a
P0	NAM	0.68±0.10b	0.32±0.03a	2.10±0.15a	1.00±0.13b
	AM	0.97±0.26a	0.43±0.01a	2.26±0.57a	1.39±0.27a
	AMF	*	NS	NS	*
Significance	P	NS	NS	NS	NS
	AMF×P	*	NS	NS	*

2.3.2 Effects of AMF on the contents of osmotic regulatory substances in the roots of *S. davidii* under low-phosphorus stress

The effect of AMF on the osmotic regulatory substances content in the roots of *S. davidii* under low phosphorus stress is shown in Table 4. Under NAM, the contents of proline, soluble sugar and soluble protein increased first and then decreased with the increase of phosphorus stress intensity, all reaching the maximum at P0.25 treatment, with levels significantly higher than that at P0 treatment (except for the soluble sugar content) ($P < 0.05$). Compared with NAM, inoculation with AMF significantly increased proline and soluble protein contents under the P0 treatment and significantly increased soluble sugar contents under the P0.25 treatment ($P < 0.05$); there were no significant differences among the other treatments ($P > 0.05$). After inoculation with AMF, the maximum contents of proline and soluble sugar were 249.86 $ug·g^{-1}$ and 63.94 $mg·g^{-1}$, respectively, in the P0.25 treatment. The soluble protein content of the P0 treatment was the largest, which was 20.72 $mg·g^{-1}$.

Table 4 Effects of AMF on the contents of proline, soluble sugar and soluble protein in the roots of *S. davidii* under low phosphorus stress

P Status	AMF Status	Proline content ($ug \cdot g^{-1}$)	Soluble sugar ($mg \cdot g^{-1}$)	Soluble protein ($mg \cdot g^{-1}$)
P0.5	NAM	201.49±85.92a	17.72±4.45b	17.29±3.06ab
	AM	209.38±16.17a	21.30±4.68b	13.35±2.69bc
P0.25	NAM	210.14±81.53a	31.99±22.59b	18.39±3.70a
	AM	249.86±27.62a	63.94±24.90a	20.03±0.59a
P0	NAM	118.88±65.39b	20.93±17.88b	12.53±1.98c
	AM	227.62±62.17a	49.05±21.13ab	20.72±1.67a
	AMF	*	*	*
Significance	P	*	NS	*
	AMF×P	NS	*	**

2.3.3 Effects of AMF on root protective enzyme activity and malondialdehyde content of *S. davidii* under low-phosphorus stress

The effects of AMF on the root protective enzyme activity and malondialdehyde content of *S. davidii* under low phosphorus stress are shown in Table 5. Without AMF inoculation, with the increase in phosphorus stress intensity, the activities of acid phosphatase, catalase (CAT) and peroxidase (POD) increased, and the activity of superoxide dismutase (SOD) first increased and then decreased. The activity of acid phosphatase and CAT in the P0 treatment was the highest and was significantly higher than that in the P0.5 treatment ($P < 0.05$). The SOD activity in the P0.25 treatment was the highest, and the POD activity in the P0 treatment was the highest, at 35105.82 $U \cdot g^{-1}$. Inoculation with AMF, phosphorus stress and the interaction of AMF×P had no significant effect on the content of malondialdehyde (MDA) ($P > 0.05$). Compared with NAM, inoculation with AMF significantly increased the acid phosphatase activity and SOD activity under the P0 treatment, significantly increased SOD activity, POD activity and CAT activity under the P0.25 treatment, and significantly increased the acid phosphatase activity and CAT activity under the P0.5 treatment ($P < 0.05$). There were no significant differences among the other treatments ($P > 0.05$). After inoculation with AMF, the activities of acid phosphatase and CAT under the P0 treatment were the highest, at 35.21 $mU \cdot g^{-1}$ and 190.82 $U \cdot g^{-1} \cdot min^{-1}$, respectively. The SOD activity and POD activity of the P0.25 treatment were the highest, at 103.68 $U \cdot g^{-1}$ and 51198.30 $U \cdot g^{-1} \cdot min^{-1}$, respectively.

Table 5 Effects of AMF on acid phosphatase activity, superoxide dismutase activity, peroxidase activity, catalase activity and malondialdehyde content of *S. davidii* under low-phosphorus stress

P Status	AMF Status	Acid phosphatase ($mU \cdot g^{-1}$)	SOD activity ($U \cdot g^{-1} \cdot min^{-1}$)	POD activity ($U \cdot g^{-1} \cdot min^{-1}$)	CAT activity ($U \cdot g^{-1} \cdot min^{-1}$)	MDA content ($nmol \cdot g^{-1}$)
P0.5	NAM	13.98±5.11c	37.69±4.05c	18498.09±3682.36c	34.71±11.15c	22.89±1.88a
	AM	20.43±5.83b	36.42±7.59c	20135.31±8240.87bc	72.75±29.32b	19.94±4.29a
P0.25	NAM	22.44±6.59b	41.77±7.01c	20759.41±7080.52bc	76.60±40.23b	20.68±3.60a
	AM	22.64±0.88b	103.68±8.33a	51198.30±4302.00a	188.27±84.23a	22.73±1.89a
P0	NAM	27.44±12.65b	26.35±6.05d	35105.82±2161.78abc	167.94±32.76a	30.13±2.89a
	AM	35.21±24.29a	82.56±4.03b	43261.65±3672.72ab	190.82±62.84a	23.97±6.23a
	AMF	*	**	*	**	NS
Significance	P	*	*	NS	**	NS
	AMF×P	*	**	*	**	NS

2.4 Effects of AMF on endogenous hormones in *S. davidii* under low phosphorus stress

The effect of AMF on the endogenous hormones of *S. davidii* under low-phosphorus stress is shown in the figure (Fig. 2). Under NAM, the contents of IAA, GA_3 and BR in roots increased first and then decreased with increasing phosphorus stress intensity, and the contents of IAA, GA_3 and BR in leaves first decreased and then increased. The contents of IAA, GA_3 and BR in roots were the largest under the P0.25 treatment and were significantly higher than those under the other treatments ($P < 0.05$). The contents of GA_3 and BR in leaves in the P0.5 treatment were the highest, at 402.33 Pg/mL and 128.46 ng/L, respectively, and were significantly higher than those in the P0 treatment ($P < 0.05$). Compared NAM, inoculation with AMF significantly increased the content of BR in roots, the content of IAA in roots and leaves under P0 treatment, the root GA_3 content in the P0 and P0.25 treatments, and the leaf GA_3 content in the P0.25 treatment (*P*

< 0.05). After inoculation with AMF, the contents of IAA in leaves and BR in roots were the highest in the P0.5 treatment, at 98.03 μg/L and 135.79 ng/L, respectively. The content of IAA in roots was the highest in the P0 treatment, at 78.86 μg/L.

2.5 Effects of AMF on nitrogen and phosphorus contents and the N:P ratio of *S. davidii* under low-phosphorus stress

Phosphorus stress had a significant effect on the nitrogen content of leaves, stems and roots of *S. davidii* seedlings ($P < 0.05$), and there was an interaction between AMF and phosphorus stress (Fig. 3 A, B, C). Without AMF inoculation, with the increase in phosphorus stress intensity, the nitrogen content of roots first decreased and then increased, the nitrogen content of stems increased, and the nitrogen content of leaves first increased and then decreased. The root nitrogen content was the largest in the P0.5 treatment, which was significantly higher than that of the other treatments ($P < 0.05$). Both stem and leaf nitrogen contents were the smallest in the P0.5 treatment. Compared with NAM, inoculation with AMF significantly increased root nitrogen contents ($P < 0.05$) (except for the P0.5 treatment), significantly decreased stem nitrogen contents in the P0 treatment and decreased leaf nitrogen content ($P < 0.05$).

Inoculation with AMF, phosphorus stress and the AMF×P interaction had a significant effect on the phosphorus content of the roots, stems and leaves of *S. davidii* seedlings ($P < 0.05$) (Fig. 3 D, E, F). When AMF was not inoculated, the phosphorus content in roots did not change significantly under low phosphorus stress, while the phosphorus content in stems and leaves decreased significantly under P0 treatment. Compared with NAM, inoculation with AMF significantly increased the phosphorus content of roots and stems in the P0.25 and P0 treatments and significantly decreased the phosphorus content of leaves in the P0 treatment (P □ 0.05).

Inoculation with AMF and phosphorus stress had a significant effect on the N:P value of leaves and roots of *S. davidii* seedlings ($P < 0.05$), and there was an interaction between AMF and phosphorus stress (Fig. 3 G, H, I). Without AMF inoculation, with the increase in P stress intensity, the N:P of roots and leaves first decreased and then increased, and the N:P of stems increased. The root N:P of the P0.5 treatment was the largest and was significantly higher than that of the other treatments ($P < 0.05$). The stem and leaf N:P in the P0 treatment was the largest and was significantly higher than that in the other treatments ($P < 0.05$). Compared with NAM, inoculation with AMF significantly reduced root and leaf N:P under the P0.5 and P0 treatments and significantly decreased stem N:P under the P0.25 and P0 treatments.

3 Discussion

The AMF colonization rate is an important indicator of whether AMF have established a symbiotic relationship with host plants. It can measure the ecological adaptability of AMF and, to a certain extent, also determines plant growth and stress resistance[29]. The results of this study showed that the low phosphorus stress treatments (inoculated or not inoculated with AMF) increased the AMF colonization rate of *S. davidii* compared with NAM. AMF inoculation significantly increased the AMF colonization rate of the roots of the *S. davidii* seedlings, which is similar to the findings in *Faidherbia albida* by Hailemariam *et al*.[30]. Mycorrhizal plants grown under low phosphorus stress are more responsive and dependent on AMF[31]. Therefore, in a low phosphorus environment, inoculation of AMF can facilitate a good symbiotic relationship with plant roots, thereby enhancing the survival of plants under stress.

3.1 Effects of AMF on the growth mechanism of *S. davidii* seedlings under low phosphorus stress

AMF are obligate symbiotic fungi, and their main function is to provide mineral elements, especially phosphorus. Phosphorus is an essential mineral element for plants, accounting for 0.2% of the dry weight of plant cells, so plant cell growth requires a large amount of phosphorus[32]. Phosphorus deficiency in soil is the main limiting factor for plant growth, and plant roots are the link between the soil and the plant itself and are the most important organ plant absorption of water and nutrients from the soil environment. A good root system is a prerequisite for plants to adapt to low phosphorus stress. During the process of sensing the changes in nutrients in the environment, roots can produce morphological and physiological changes to cope with environmental stress[33]. Leaves are plant vegetative organs, and their function is to carry out photosynthesis to synthesize organic matter and to facilitate transpiration, providing the root system with the power to absorb water and mineral nutrients from the outside world[34]. From this experiment, without AMF inoculation, root dry weight, root-shoot ratio, total root length, root surface area, root tip number, and root hair number showed an upward trend with the aggravation of low-phosphorus stress. The total root length, root surface area, root tip number and root hair number all reached maximum values under the P0.25 treatment, with levels significantly higher than those under the P0.5 treatment. The plant height and aboveground dry weight decreased gradually with the intensification of low-phosphorus stress. Ting *et al* found that the phosphorus-efficient *Fagopyrum tataricum* variety had higher root vigor, root biomass and more developed root systems, which was consistent with the results of this study[35]. This is because under low phosphorus stress conditions, to obtain the phosphorus nutrient elements needed for growth, *S. davidii* transports more carbohydrates to the roots, increases the biomass of underground roots, increases the root-shoot ratio, promotes root growth, and forms a well-developed root system by increasing root length, total root surface area, and number of root hairs, guaranteeing effective phosphorus absorption to plants[36]. Compared with NAM, inoculation with AMF significantly reduced the root dry weight, root-shoot ratio, total root length, root surface area, root tip number and root hair number under the P0.25 treatment and significantly increased the total root length, root tip number, plant height, growth rate, and aboveground biomass of *S. davidii* under the P0 treatment. After inoculation with AMF, *S. davidii* roots formed symbiosis with AMF, and the roots provided nutrients for the mycorrhizal hyphae, which reduced the growth of their own root system and reduced the root biomass; however, with the continuous reduction in phosphorus treatment concentration, AMF can increase total root length and root tip number to increase nutrient uptake to maintain the high biomass of *S. davidii* under low phosphorus stress, ultimately increasing the growth rate[37]. The promoting effect of AMF on aboveground biomass accumulation and plant height formation under low phosphorus stress was similar to the research results in other plants[38].

3.3 Effects of AMF on the physiological mechanisms of *S. davidii* seedlings under low phosphorus stress

Chlorophyll a and chlorophyll b are the most important pigments involved in photosynthesis and have the functions of absorbing, transmitting and transforming light energy. Within a certain range, the chlorophyll content is proportional to the photosynthetic rate, which directly reflects the level of plant photosynthetic capacity[39]. In this experiment, under NAM, low-phosphorus stress reduced the contents of chlorophyll a, chlorophyll b and total chlorophyll in the leaves of *S. davidii*. Compared with NAM, inoculation with AMF significantly increased chlorophyll a and total chlorophyll contents in the P0.25 and P0 treatments, similar to the findings in *Chili* by Elahi *et al*.[40]. Studies have shown that the increase in chlorophyll content may be related to the absorption of phosphorus and magnesium by AMF[41]. In our study, inoculation with AMF significantly increased the leaf phosphorus content in the P0 treatment compared with that in NAM. More phosphorus and chlorophyll contents in leaves provide the basis for maintaining higher photosynthetic capacity.

Plants show a series of physiological adaptation mechanisms under low phosphorus to adapt to these adverse environmental conditions[42]. In this study, when AMF was not inoculated, the contents of proline, soluble sugar and soluble protein in roots increased first and then decreased with the increase in phosphorus stress intensity, which shows that under low phosphorus stress, the osmotic potential of the plant under stress is reduced by these substances, the osmotic potential of the cell is maintained, and the cell is protected so that the plant can adapt to the adversity; however, if the intensity of low phosphorus stress is too high, these osmotic regulators will be destroyed, and their regulatory capacity will be reduced[43]. Compared with NAM, inoculation with AMF increased the osmotic regulators, such as proline, soluble sugar and soluble protein, in roots to a certain extent, all of which were significantly increased under the P0 treatment. This is similar to previous study results[44]. This indicates that AMF symbiosis can induce changes in the secondary metabolism of *S. davidii* under low phosphorus stress, increase the biosynthesis of phytochemicals and increase the content of osmo-regulatory substances[45].

Acid phosphatase is an enzyme induced by plant roots according to the amount of external phosphorus. When external phosphorus is deficient, the activity of acid phosphatase in plants increases, thereby increasing the effective phosphorus concentration in the rhizosphere[46]. In this study, when AMF was not inoculated, the acid phosphatase in the roots was significantly increased under the low phosphorus environment and reached the maximum value under the P0 treatment, which is similar to the findings in soybean by NADIRA *et al*.[47]. Compared with NAM, inoculation with AMF significantly increased the acid phosphatase activity in roots (P0 and P0.5 treatments), possibly because of the symbiotic colonization between AMF and plant roots in root cortex cells to obtain the required carbohydrates; at the same time, mineral nutrients such as N, P, and K can also be transferred from the soil to the root cortex and secrete phosphatases from organophosphorus compounds to hydrolyze phosphate[48].

Superoxide dismutase, peroxidase and catalase are key enzymes involved in plant stress resistance in the protective enzyme system; they can scavenge the oxygen free radicals generated by the disturbance in plant tissues through oxidation, thereby reducing damage to plants and protecting plants[49]. In this study, under NAM, the activities of superoxide dismutase, peroxidase and catalase in *S. davidii* roots increased to a certain extent under low phosphorus stress; in particular, catalase and peroxidase activities continued to rise, which is similar to the findings in wheat by Wang *et al*.[50]. Compared with NAM, AMF inoculation significantly increased superoxide dismutase (P0.25 and P0 treatment), oxidase (P0.25 treatment) and catalase activities (P0.5 and P0. 25 treatment), which shows that the symbiosis between AMF and *S. davidii* can improve the activity of protective enzymes under low-phosphorus stress, enhance the adaptation to a low-phosphorus environment, and maintain a stable biomass[51].

Malondialdehyde is the product of membrane lipid peroxidation. When plants are under stress, the accumulation of malondialdehyde increases, which can aggravate cell membrane damage and damage membrane lipids[52]. In this experiment, compared with NAM, inoculation with AMF had no significant effect on malondialdehyde contents in roots because the inoculation of AMF in this study increased the osmotic regulators, such as proline, soluble sugar and soluble protein; moreover, AMF can improve the activities of key protective enzymes in plant stress resistance reactions, such as POD, SOD and CAT, to a certain extent to remove oxygen free radicals in plant tissues and reduce the damage to plants under stress.

3.4 Effects of AMF on the endogenous hormones of *S. davidii* seedlings under low-phosphorus stress

Endogenous hormones, as important regulators of plant metabolism, are involved in a series of physiological and biochemical processes[53]. In this experiment, when AMF was not inoculated, low phosphorus stress (P0.25 treatment) significantly decreased the contents of BR, GA_3 and IAA in leaves and significantly increased the contents of BR, GA_3 and IAA in roots. This is because under a low phosphorus environment, plants can induce root structure changes and improve phosphorus utilization efficiency by transporting the accumulated GA_3, BR and IAA in leaves to roots[54]. Compared with no inoculation, inoculation with AMF significantly increased leaf IAA contents in the P0.5 and P0 treatments, significantly increased root IAA content in the P0 treatment, significantly increased root GA_3 and BR contents under the P0.25 and P0 treatments, and significantly decreased leaf GA_3 and BR contents under the P0.5 and P0 treatments. It has been reported that inoculation with AMF significantly increased the content of IAA in roots and leaves of *Catalpa bungei* C.A.Mey., significantly increased the content of GA_3 and BR in roots, and significantly decreased the content of GA_3 and BR in leaves to regulate plant growth and improve stress resistance[55].

3.5 Effects of AMF on the mineral elements of *S. davidii* seedlings under low-phosphorus stress

Nitrogen is an essential element for plant growth. It is an important part of plant proteins and related enzymes and plays a major role in plant growth. In this study, when AMF were not inoculated, low phosphorus stress significantly decreased the nitrogen content in roots and increased the nitrogen content in stems and leaves, while the changes in nitrogen content in leaves were smaller than those in roots and stems. This is similar to the findings in *Zea mays L*. by Rafique *et al*.[56]. This may have been due to the slow growth of plant cells due to the lack of phosphorus, the increase in chlorophyll content, and the increase in nitrogen content in leaves and stems to maintain growth[57]. The fact that low phosphorus stress significantly reduces the nitrogen content of roots is because low phosphorus stress reduces the biomass of plant roots, thereby reducing the ability of plant roots to obtain nitrogen from the outside world. On the other hand, nitrogen is particularly important for plant photosynthesis. Under external stress, to maintain normal growth, plants may choose to transport

more nitrogen to the aboveground parts to meet the needs of photosynthesis for nitrogen[58]. Compared with no inoculation, inoculation with AMF significantly increased the nitrogen content in roots (P0.25 and P0 treatments) and decreased the nitrogen content in leaves and stems. This is because AMF can help plant roots obtain nitrogen from the soil, so plants will allocate more carbohydrates to AMF and then obtain more N through AMF, resulting in an increase in the nitrogen content of plant roots, which is similar to the findings in *Lolium multiflorum* by Liu *et al*.[59]. In addition, inoculation with AMF increased the adaptation of plants to adversity and promoted the growth of the aerial parts of plants, resulting in a concentration dilution effect, which reduced the nitrogen contents of stems and leaves[60].

Phosphorus is also one of the essential nutrients for plant growth, and it is the second most important nutrient after nitrogen that limits crop growth. This nutrient is involved in a range of plant processes, such as photosynthesis, respiration, energy production, and nucleic acid biosynthesis, and is a component of some plant structures, such as phospholipids[61]. In this study, when AMF was not inoculated, low phosphorus stress significantly decreased the phosphorus content of leaves and stems (P0 treatment). This is similar to the findings in *Zea mays L*. by Rafique *et al*.[56], and occurred because the phosphorus in the plant is obtained from the external environment through the root system. Under phosphorus deficiency, the growth of the plant is inhibited, which reduces the acquisition of phosphorus in the soil by the plant; therefore, the phosphorus content in plant leaves and stems decreases, and when phosphorus is deficient, plant photosynthetic products are preferentially distributed to the underground parts, especially the root tips, to obtain phosphorus, so the phosphorus content of the roots remains unchanged. Compared with NAM, inoculation with AMF significantly increased the phosphorus content of roots and stems (P0.25 and P0 treatments) and significantly increased the phosphorus content of leaves (P0 treatment). Relevant studies have shown that AMF can absorb phosphorus elements within 10 cm of the soil surface through extra root hyphae and then transport the absorbed phosphorus elements to root epidermal cells, where these elements are eventually absorbed by plant cells[62]. AMF can significantly improve the uptake of phosphorus by plants, especially in phosphorus-deficient environments[63]. Under a phosphorus stress environment, the reduction in phosphorus contents in plant leaves and stems will have a significant impact on plant growth, affecting photosynthesis and other physiological processes. Inoculation with AMF can alleviate this adverse effect of phosphorus stress and enhance the adaptation of *S. davidii* seedlings to phosphorus stress.

The N:P of plant leaves is important for predicting the nutrient limitation of plants. Generally, plants with an N:P ratio less than 14 are in a nitrogen-limited state, and plants with an N:P ratio greater than 14 are in a phosphorus-limited state[64]. Our study showed that without AMF inoculation, the N:P ratio of leaves of *S. davidii* seedlings under phosphorus stress increased from 17.6 to 18.6, indicating that low phosphorus stress significantly aggravated plant phosphorus limitation[65]. In this study, inoculation with AMF decreased the N:P of leaves, stems and roots compared with those under NAM. Inoculation with AMF can expand the absorption area of plant roots through extraroot hyphae, thereby enhancing the ability to compete for nutrients and reducing the N:P ratio of plants. According to the growth rate hypothesis, living organisms need to make relatively large investments in phosphorus-rich ribosomes and rRNA to support rapid protein synthesis associated with rapid growth. The element stoichiometry leading to fast-growing individuals or taxa would be skewed toward phosphorus, so fast-growing organisms would exhibit lower N:P and C:P ratios[66]. Inoculation with AMF maintained rapid growth under phosphorus stress by reducing the N:P ratio of plants. In addition, our study also found that inoculation with AMF could maintain a constant N:P ratio in leaves under this phosphorus stress. This indicated that the regulation of plant body N:P reached a balance, which could promote the growth of *S. davidii* seedlings.

4. Conclusion

The plant height, growth rate, aboveground dry weight and chlorophyll content of *S. davidii* were significantly decreased under low-phosphorus stress. Under certain low phosphorus stress conditions (P0.25 treatment), *S. davidii* showed improved root morphology, increased contents of osmotic regulatory substances, and increased activity of protective enzymes and contents of hormones to adapt to low phosphorus stress. However, when the low phosphorus stress intensity was further increased under the P0 treatment, the regulatory effect was severely weakened. After inoculation with AMF, the roots and AMF formed a symbiosis, although the growth of the root system and the root biomass were reduced; however, the total root length, root tip number and growth rate under the P0 treatment were significantly improved along with the chlorophyll a, total chlorophyll, proline, soluble sugar, and soluble protein contents and acid phosphatase activity, superoxide dismutase activity, and IAA content, while the N:P of leaves was reduced and stems and roots maintained a higher biomass under low phosphorus stress. Therefore, for ecological restoration and forage improvement using *S. davidii* plantings in acidic soils in the karst region of Southwest China, inoculation with AMF may be a good strategy to stabilize the yields of *S. davidii*.

Declarations

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Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Conflicts of Interest

All authors declare that they have no confict of interest.

Contribution

KKC, LTW and LLZ conceived and designed research. KKC, LTW, LZ, and YYL conducted experiments. KKC, LTW, HS and PCW analysed the data. KKC and LTW wrote the manuscript. All authors read and approved the manuscript.

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Figures

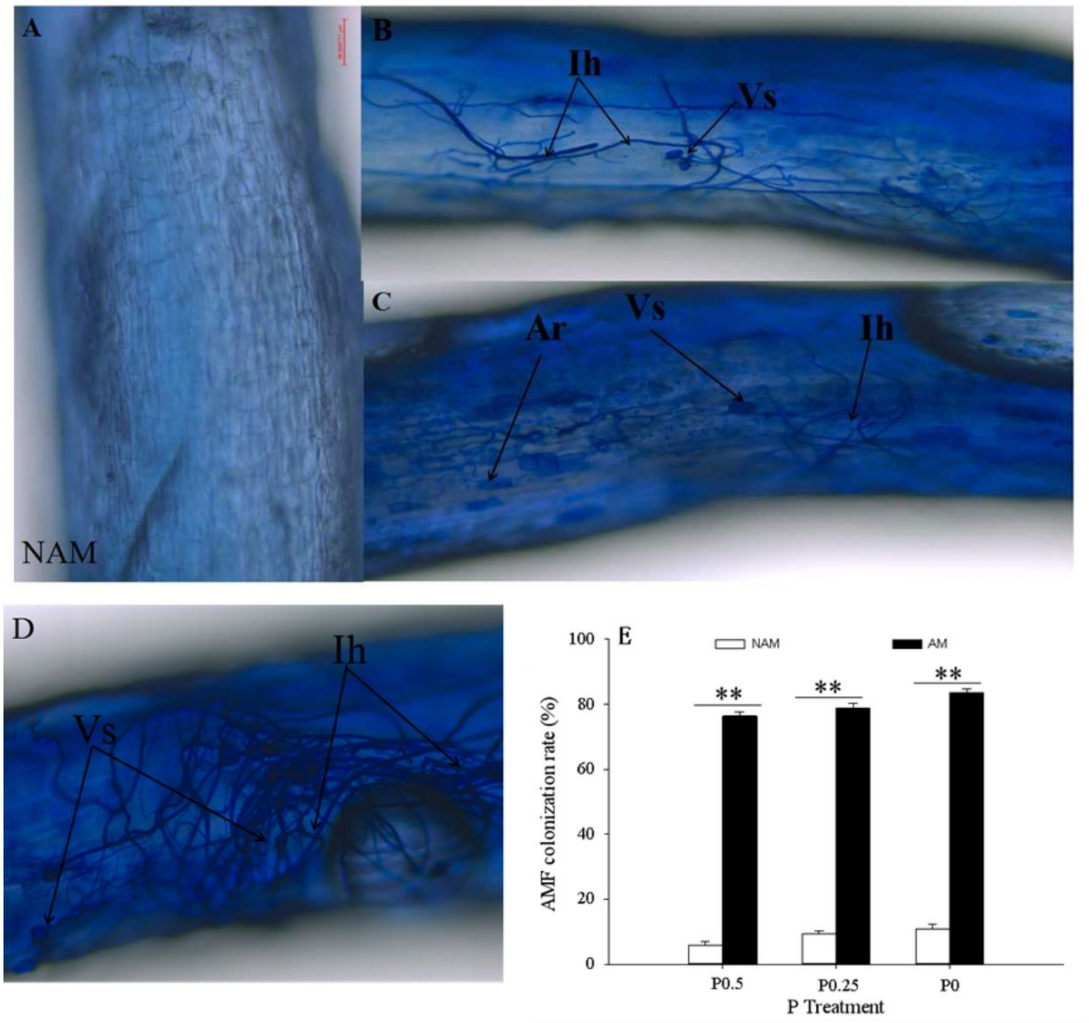

Figure 1

Development of arbuscular mycorrhizal fungus (AMF) in *S. davidii* seedling roots visualized by Trypan blue staining. (A) The root of a noninoculated plant. (B) Inoculated roots under the P0.5 treatment, (C) P0.25 treatment, and (D) P0 treatment, and the (E) colonization rate of mycorrhizal *S. davidii* seedlings. Ih, intraradical hyphae; Ar, arbuscule; vs., vesicles; (A, B, C and D, G: ×400). NAM, non-AMF-inoculated; AM, AMF-inoculated. Colonization rate** means P < 0.01.

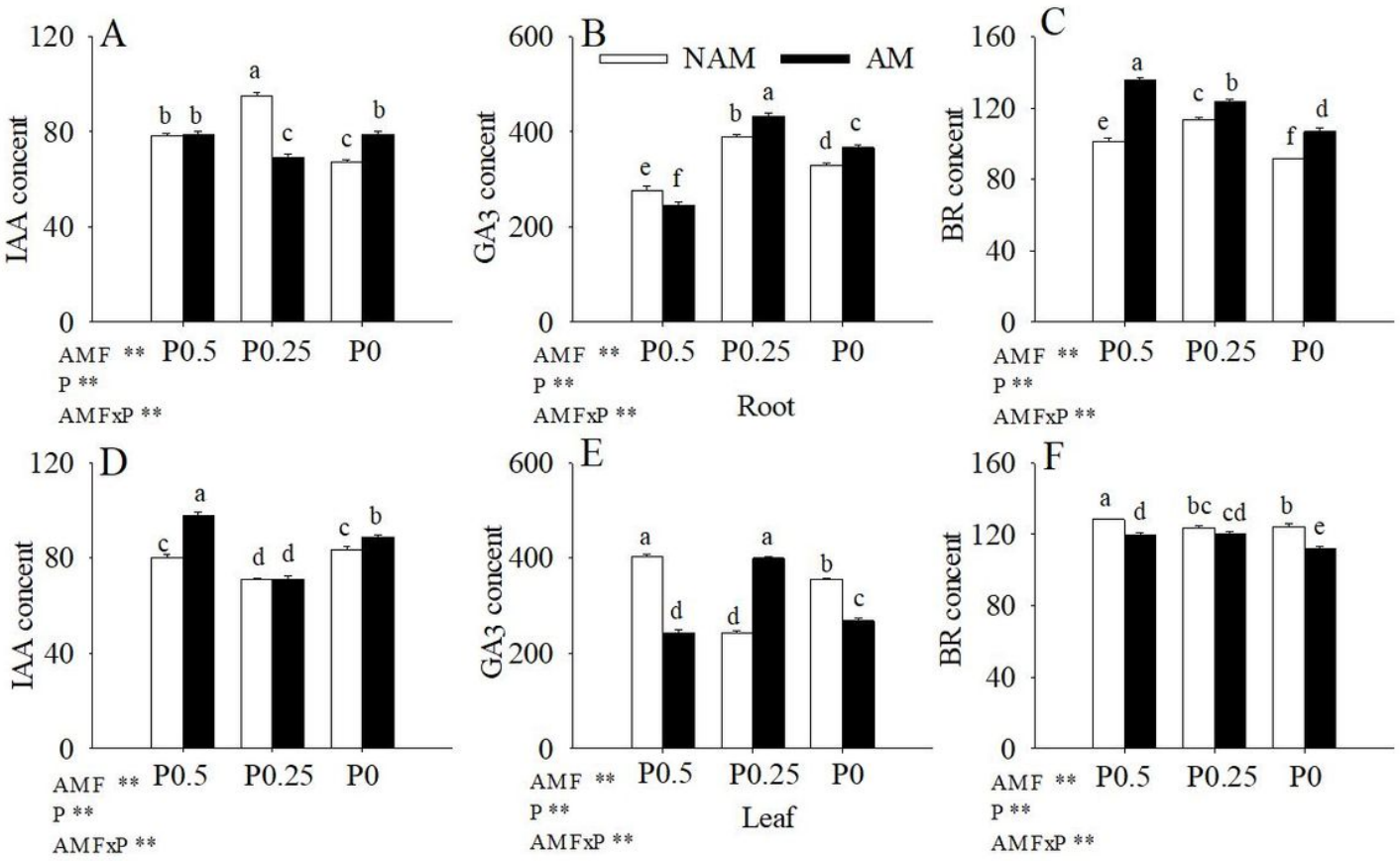

Figure 2

Effects of AMF on endogenous hormones of *S. davidii* under low phosphorus stress (values are means ± SE). Different lowercase letters indicate significant differences at $P < 0.05$. NAM, non-AMF-inoculated; AM, AMF-inoculated. AMF×P, interaction between AMF inoculation and phosphorus stress. *ns*, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$.

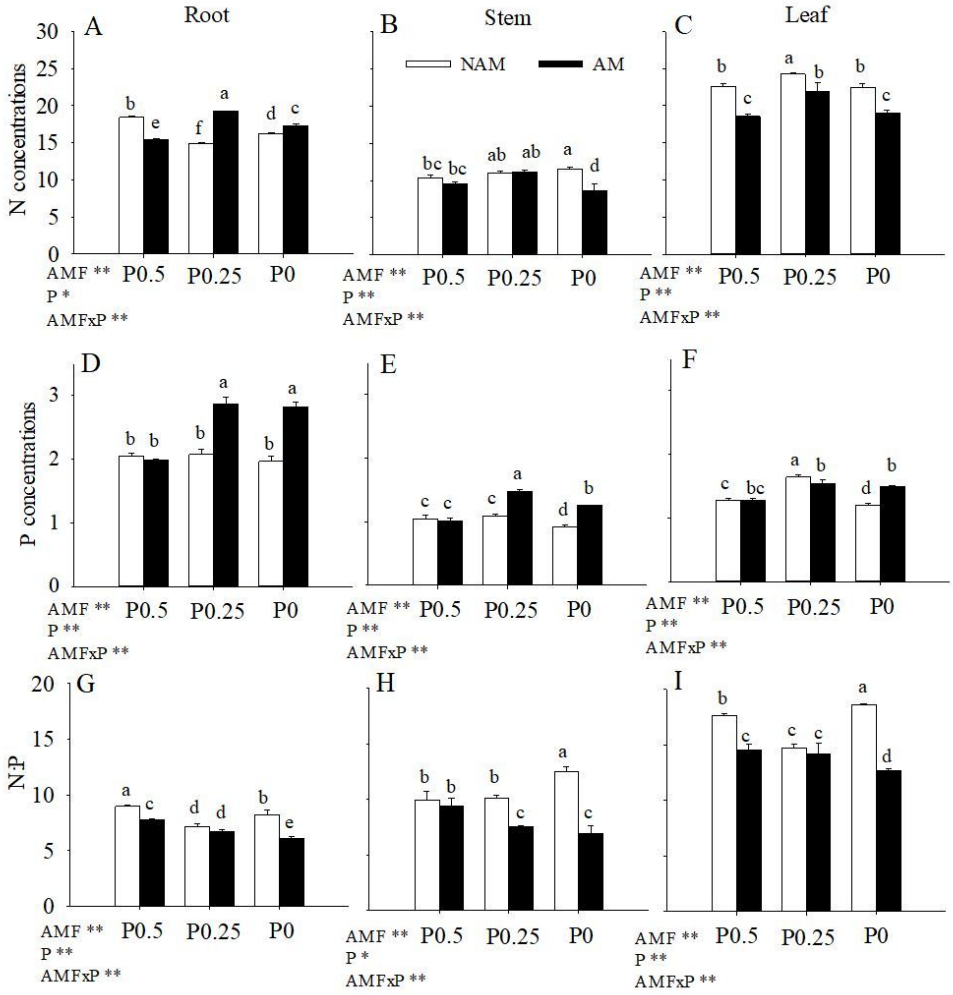

Figure 3

Effects of AMF on nitrogen and phosphorus contents and N:P in leaves, stems and roots of *S. davidii* seedlings under low phosphorus stress (values are means ± SE). Different lowercase letters indicate significant differences at $P < 0.05$. NAM, non-AMF-inoculated; AM, AMF-inoculated. AMF×P, interaction between AMF inoculation and phosphorus stress. *ns*, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$.