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Rhizophagus intraradices improves arsenic tolerance in *Sophora viciifolia* Hance

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Abstract

Purpose: Arbuscular mycorrhizal fungi (AMF) are capable of protecting host plants against heavy metal toxicity, whereas rare knowledge has been acquired on the underlying effects of AMF on woody leguminous species under arsenic (As) stress. This study was aimed that whether AMF inoculation improves the As tolerance in *Sophora viciifolia* (a king of leguminous shrub).

Methods: *S. viciifolia* seedlings were inoculated with AMF *Rhizophagus intraradices*, and then the seedlings were grown at three levels (0, 50, and 100 mg As kg^{-1} soil) of As-polluted soil by performing the potted experiments. The plant growth, photosynthetic parameter, oxidative damage, antioxidant enzyme activities and gene expression of phytochelatin synthase in *R. intraradices*- and non-inoculated *S. davidii* seedlings under the different levels of As stress were analyzed.

Result: Compared with non-inoculated *S. viciifolia* at the identical As level, *R. intraradices*-inoculated *S. viciifolia* were higher in the shoot and root dry weight, plant height, root length, photosynthetic rate, stomatal conductance, transpiration rate, maximal photochemical efficiency of PSII photochemistry, actual quantum yield, and photochemical quenching values, while the intercellular CO_2 concentration and non-photochemical quenching values were lower. As-induced oxidative stress generating malondialdehyde, hydrogen peroxide and superoxide in the *S. viciifolia* leaves and roots reduced significantly by *R. intraradices* inoculation, whereas the activities of antioxidative enzymes (e.g., superoxide dismutase, peroxidase, and catalase) in *S. viciifolia* leaves and roots were increased by *R. intraradices* inoculation. Notably, *R. intraradices* inoculation up-regulated the gene expression of *S. viciifolia* phytochelatins in the leaves and roots.

Conclusion: These results demonstrated that *R. intraradices* inoculation enhanced the As tolerance in *S. viciifolia* seedlings by improving the plant growth, gas exchange, chlorophyll fluorescence, reactive oxygen species, antioxidant enzymes and gene expression of *S. viciifolia* phytochelatins. The present study verified a multifarious positive role of AMF for woody leguminous species under As stress.

Keywords: Arbuscular mycorrhizal fungi, *Sophora davidii*, Arsenic stress, Photosynthesis, Reactive oxygen species, *PCS1* gene expression

Introduction

Arsenic (As) is an ubiquitous "heavy metal" (HM) element in the earth crust (Li et al. 2019), and the normal As content in soil and water does not exceed 10 mg kg^{-1} (Gomes et al. 2015; Li et al. 2018). Over the past decades, As accumulation in agricultural products beyond the threshold values has seriously jeopardized human health through food chains in many places on earth (Li et al.

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2019). As contamination has been progressively worse as impacted by excessive anthropogenic activities (Das et al. 2017a, b; De Andrade et al. 2015; Sharma et al. 2017; Zhang et al. 2017). Excess As in the terrestrial ecosystem have a negative influence on the growth and development of higher plants (Li et al. 2019). As ions in the natural ecosystems are often absorbed into the root hair cells by the non-specific transporters of plasma membrane (Gomes et al. 2015; Luo et al. 2016). As in plant cells suppresses the cytoplasmic enzymatic activities, and then superfluous free radicals (FR) and reactive oxygen species (ROS) are subsequently formed in plant tissues (Sharma et al. 2017; Srivastava et al. 2007). As a result, the oxidative damages of cell structures, suppression of photosynthesis, reductions in plant growth and yield are caused with increasing oxidation and radical chain reactions, and vegetation degradation ultimately occur in some As-contaminated areas (Das et al. 2017a, b; De Andrade et al. 2015; Sharma et al. 2017; Spagnoletti et al. 2017).

Higher plants possess the detoxification and repair system to improve the As tolerance. The antioxidative enzymatic system, that produces antioxidant enzymes, is capable of scavenging FR and ROS to prevent oxidative damage in plant cells (Srivastava et al. 2007). Moreover, plants induce the adaptive detoxification mechanisms of phytochelatins (PCs) to alleviate HM stress (Li et al. 2019). PCs chelate HM ions (e.g., Pb, Cd, Hg, Cu, Cr, and As), and the formative stable PC-HM compounds are subsequently transported from the cytosol to the vacuoles of plant cell, accordingly, the HM toxicity can be neutralized and alleviated (Das et al. 2017a, b; Xu et al. 2014). In recent years, *PCS* genes in some higher plants are isolated and described, and the overexpression of Arabidopsis *PCS* genes in other plant species are proved to directly increase the HM tolerance (Li et al. 2019).

Arbuscular mycorrhizal fungi (AMF) are capable of developing symbiotic relationships with over 80% of terrestrial higher plants, which include those growing in As-contaminated environments (Sharma et al. 2017; Shi et al. 2017; Smith and Read 2008). AMF are critical as a "bridge" between plants and rhizosphere soil, they acquire carbohydrate compounds and lipids from host plants, and in return transfer mineral nutrients from rhizosphere soil to host plants via their arbuscules and hyphal coils, when host plants cannot acquire easily (Gong et al. 2013; Riley and Corradi 2013). The widely recognized mechanism on AMF improving the As tolerance in host plants is the "growth dilution effect," which indicates that AMF assist host plants to improve the growth and to keep the higher phosphate/arsenate ratios in plant tissues (Li et al. 2018; Spagnoletti and Lavado 2015; Zhang et al. 2020). *Hymenoscyphus ericae*, an ericoid mycorrhizal fungi, helps *Calluna vulgaris* to maintain low As levels in host plants by inhibiting cellular arsenic to arsenite and excluding arsenite from plant cells (Sharples et al. 2000). It is also proved that AMF can facilitate the high-affinity P/As transfer into *Holcus lanatus* roots (Gonzalez-Chavez et al. 2002). AMF symbiosis also improve the As tolerance in host plants by regulating some gene expression of host plants. AMF (*Glomus intraradices*) inoculation downregulates the expression of the *HvPht1*gene (encoding high-affinity inorganic orthophosphate (Pi)-uptake systems directly via root epidermis and root hairs) in *Hordeum vulgare*, thus, decrease the uptake of As in barley roots of *H. vulgare* (Christophersen et al. 2009). *G. intraradices*-induced expression of *GiPT* (high-affinity Pi/As transporter) gene shows a correlation with As uptake within the external hyphae (González-Chávez et al. 2011). Whereas necessary information regarding the gene expression of *PCS* in AMF-inoculated plants under As stress has been scarce.

Sophora viciifolia Hance. is a type of vigorous perennial leguminous shrub. Such species is characterized by its developed root system, high survival rate, fast growth rate, and prominent adaptability to harsh environments (e.g., nutrient depletion, drought, salinity and HM) (Das et al. 2017a, b; Xu et al. 2014). Pb toxicity for *S. viciifolia* seedlings is alleviated by the AMF (*Funneliformis mosseae*) symbiosis, and the *F. mosseae* inoculation upregulates the expression of *SvPCS1* gene in the roots under Pb stress (Xu et al. 2014). *S. viciifolia* has been extensively distributed in warm-temperate to subtropical areas of China, and these areas are generally polluted by As in different degrees. However, rare information on the AMF-inoculated *S. viciifolia* seedlings under As stress has been reported. In the present study, a pot experiment was performed to explore the effects of *Rhizophagus intraradices* on the plant growth, photosynthesis, reactive oxygen species, antioxidant enzymes, and genetic expression of *SvPCS1* in *S. viciifolia* seedlings at the different levels of As stress. The aim was to determine the related physiological and molecular mechanisms on AMF improving the As tolerance in *S. viciifolia* seedlings. If AMF-inoculated *S. viciifolia* seedlings have the better growth performance than non-inoculated seedlings under As stress, AMF-inoculated *S. viciifolia* might be considered an ideal pioneer tree species in As-contaminated soil.

Materials and methods

Experimental design

The experiment of this study consisted of six treatments, which was set in a complete randomized block design with two factors below: (1) AMF treatments, i.e., *R. intraradices* and non-AMF inoculated control; (2) three available As concentrations in soils, i.e., 0, 50, and 100 mg As/kg dry soil. By complying with the established standard

for the grade of As pollution in Risk Control Standard of Soil Pollution in Agricultural Land in China (GB 15618-2018), when the As content in farmland soil exceeded 100 mg/kg, it would be considered high risk, and the soil was forbidden to plant agricultural products. Besides, when the 50 mg/kg As content in soil was identified as a middle-level pollution, agricultural products would be controlled by Food and Drug Administration. Each of the six treatments contained three replicates, so there were a total of 18 pots (one seedling per pot).

Growth substrate

Farmland topsoil (5–20 cm) was collected from the campus of the Henan University of Science and Technology (HAUST), Henan Province, China. Subsequently, the soils were mixed with the sand and organic matter (soil, sand, and organic matter at 3:1:1, v/v/v). The physicochemical properties of mixed soil were as follows:, organic matter 50.52 g kg^{-1}, available potassium 83.35 mg kg^{-1}, available nitrogen 40.52 mg kg^{-1}, Olsen phosphorus 8.17 mg kg^{-1}, and pH 7.8 (1:5 soil:water ratio), and the extractable metal concentrations in soils were as follows: As 5.34, Fe 3.16, Mn 2.17, Cu 0.14, and Zn 0.98 $mgkg^{-1}$. 5.65 g Na_3AsO_4 •12 H_2O dissolved in 1 L pure water, and then 0, 50 and 100 mL arsenic solutions were respectively introduced into per 1 kg dry soil mixture. Then, the mixture was stirred completely with the blender. Lastly, three available As concentrations in soils (0, 50, and 100 mg As per kg dry soil) were prepared. For the mentioned potted experiment, the soil mixture was autoclaved for 2 h at 121 °C and 0.11 MPa prior to the application.

Plant material and growth conditions

Sophora viciifolia seeds were collected in November 2016 from Shimen Realgar Mine (N 29°38′32″, E 111°2′17″), Hunan Province. This mine acted as the largest producer of realgar in China. Plump *S. viciifolia* seeds were treated with 75% ethanol for 15 min, washed with purified water, and then sowed in autoclaved wet sand at 28 °C. In the previous study of the authors, the root morphological characteristics of *S. viciifolia* seedlings in conical frustum plastic containers containing 2 kg of soil mixture were suggested to have no obvious difference with those grown in field for three months (Zhang et al. 2020). After growing for 20 days, healthy seedlings were transplanted into conical frustum plastic containers with 2 kg of soil mixture contained. *S. viciifolia* received the cultivation in a solar greenhouse, the average temperature ranged from 15 to 25 °C, and the temperature was regulated by using the ventilation system and the thermal insulation quilt. The relative humidity of the growth chamber ranged from 50% to 80% from April to June 2016. All the treatments received a nutrient supplement of 500 mL Hoagland's solution (1.0 mmol/L NaH_2PO_4) (Hoagland and Arnon 1950). To avoid the inhibition of AMF symbiosis by excessive P in soils, 50 mL modified Hoagland solution (containing only 25% of P, 0.25 mmol/L NaH_2PO_4) was supplemented weekly, and the soil moisture was maintained at a field capacity of 70% by adding a certain amount of deionized water.

Inoculation treatment

AMF strain *Rhizophagus intraradices* (BGC BJ09) (N.C. Schenck & G.S. Sm.) C. Walker and A. Schüßler originated from the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China. This AMF strain was proved to facilitate the plant growth, root morphology and phytohormone balance of *Robinia Pseudoacacia* in arsenic-contaminated soils (Zhang et al. 2020). Mycorrhizal inocula comprised a mixture of AMF spores (the spore density of 350 per 10 g dry sand soil), mycorrhizal hyphae, *R. intraradices*-infected clover root segments (average 73% AMF colonization rate), as well as sandy soil. During the seedling transplantation, the respective pot in the mycorrhizal treatment was inoculated with 30 g *R. intraradices* inoculumat a soil depth of 3–4 cm, and the control non-inoculated plants received 30 g of heat-sterilized inoculum (autoclaved at 121 °C for 30 min) plus 50 ml of microbial filtrate (0.45 μm pore size) to provide a similar microflora except for AM fungus.

AMF colonization rate

The AMF colonization in plant roots was detected by using 1-cm-long root fragments. The collected root fragments were washed with deionized water, soaked in 10% KOH at 90 °C for 15 min, decolorized in alkaline hydrogen peroxide (3 mL NH_4OH, 30 mL 10% H_2O_2, 60 mL H_2O) for 20 min, and then acidified in 1% HCl and stained with 0.05% (w/v) trypan blue in lactophenol (Phillips and Hayman 1970). They were observed under the Microscope, and the AMF and arbuscules colonization rate was determined by using the grid-line intersect with the method presented by Giovannetti and Mosse (Giovannetti and Mosse 1980).

Plant measurement

After growing for three months, the physiological and biochemical parameters of *S. viciifolia* were analyzed. *S. viciifolia* seedlings were harvested. Soil which adhered to the root surface was removed with deionized water. A ruler was adopted to measure the plant height and the root length. Shoots and roots were divided to determine the separate fresh weights, and then they were weighed

after receiving the oven drying at 70 °C for 48 h to obtain the dry weight.

As and P content

As and P content in the dry roots and leaves of *S. viciifolia* seedlings were extracted through the nitric acid digestion at 270 °C, which were determined with a graphite furnace atomic absorption spectrophotometer (PerkinElmerAnalyst400, Norwalk, CT, USA) based on USEPA Method 7060A (Li et al. 2018).

Chlorophyll content

The total chlorophyll content was analyzed by complying with the method of Srivastava and Sharma (Ahmed et al. 2006). One gram of fresh leaves were crushed in 100 mL 80% acetone in ice-bath. The extracted solution was centrifuged at 2000×*g* for 10 min. The absorbance of supernatant was measured spectrophotometrically at 645 nm and 663 nm with a spectrophotometer (Shanghai Jinghua 752). The chlorophyll content was expressed in terms of mg chlorophyll present/g fresh weight of tissues.

Gas exchange and chlorophyll fluorescence

Using a portable photosynthesis system LI-6400 (LI-COR, Lincoln, NE, USA), the net photosynthetic rate (Pn), the stomatal conductance (gs), the intercellular CO_2 concentration (Ci), and the transpiration rate (E) were determined on the fifth expanded leaf of the respective plant. The analysis was performed at 2000 μmol m^{-2} s^{-1} active radiation, 350 cm^3 m^{-3} CO_2 concentration, 25.0 °C leaf temperature and 0.5 dm^3 min^{-1} atmospheric flow rate between 9:30 and 11:00 a.m. during the data acquisition (Zhang et al. 2017).

The chlorophyll fluorescence parameters were determined with a modulated PAM-2000 portable fluorometer (Imaging-PAM, Walz, Germany) on the fifth expanded leaves of *S. viciifolia*. The leaves were adapted in dark for 1 h, and then the measurements were performed between 9:30 and 11:00 a.m. at ambient temperatures. The leaves were saturated with pulse flashes of white light (2000 μmol m^{-2} s^{-1} for 3 s), and for dark-adapted leaves, the Fo (minimum fluorescence) and Fm (maximal fluorescence) were measured. Besides, for light-adapted leaves, the Fs (steady-state) and Fm′ (maximal) fluorescence were obtained. The Fo′ (minimal fluorescence level in the light-adapted state) was achieved by illuminating the leaves with a 3-s flash of far-infrared light (5 μmol m^{-2} s^{-1}). By complying with the method of Genty et al. (1989), the maximum quantum yield of the PSII photochemistry ((Fm−Fo)/Fm) and the actual quantum yield of PSII electron transport ((Fm′-Fs)/Fm′) were determined (Genty et al. 1989). The quenching due to non-photochemical dissipation (NPQ=(Fm-Fm′)/Fm′) and the coefficient of photochemical quenching (qP=(Fm′-Fs)/(Fm′-Fo′)) were calculated based on the methods described previously (Maxwell and Johnson 2000).

Measurement of oxidative damage

Fresh leaves or roots (1 g) were homogenized in 10 mL 10 mM sodium phosphate buffer (pH 7.4) on an ice bath, and then the homogenate was centrifuged at 4000×*g* for 10 min. The malondialdehyde (MDA) content was determined with the method presented by Janero (1990). The rates of H_2O_2 and $O_2^{\bullet-}$ productions were determined with the method previously published by Wang and Luo (1990). The absorbance of H_2O_2 in the assay mixture was spectrophotometrically determined at 390 nm. To analyze the $O_2^{\bullet-}$ content, 1 mL 17 mM sulfanilic acid and 1 mL 7 mM α-naphthylamine were introduced in 1 mL of the mixture for 20 min at 25 °C, and then 3 mL anhydrous was adopted to leach chlorophyll. The concentrations of $O2^{\bullet-}$ in the assay mixture were spectrophotometrically measured at 530 nm (Elavarthi and Martin 2010).

Determination of antioxidant enzymes

To extract the antioxidant enzymes, the following steps were performed under ice-cold conditions. One gram of fresh leaves or roots was homogenized in 5 mL 0.1 M cold Tris-HCl buffer (pH 7.6), and the supernatant fraction was employed after being centrifugated at 10,000×*g* for 20 min. The SOD activity (EC 1.15.1.1) was assayed spectrophotometrically at 560 nm with the method of Giannopolitis and Ries (Giannopolitis and Ries 1977). The amount of enzymes causing a 50% decrease in SOD-inhibitable photochemical reduction of nitroblue tetrazolium (NBT) was defined as 1 U SOD activity. With the method of Aebi (Aebi 1984), CAT (EC 1.11.1.6) activity was measured spectrophotometrically at 240 nm. A unit of CAT enzyme activity was expressed as the extinction coefficient of 1 μmol H_2O_2 oxidized mg^{-1} protein min^{-1}. POD (EC 1.11.1.7) activity was assessed based on the method of guaiacol oxidation (Britton and Maehly 1955). POD was quantified spectrophotometrically at 470 nm, in which 1 U POD enzyme activity was the number of grams of tetraguaiacol formed per min (Zhang et al. 2017).

RNA extraction and cDNA synthesis

The total RNA was extracted from the fresh leaves and roots with Plant Total RNA Isolation Kit (Sangon Biotech, Shanghai, China) by complying with the manufacturer's instructions. To remove the residual genomic DNA, the TURBO DNA-free kit (Applied Biosystems/Ambion) was applied, and the RNA quantity was detected with a NanoDrop 2000 (Thermo Scientific, Pittsburgh, PA, USA). The complementary DNA (cDNA) was reversely

transcribed by employing a PrimeScript RT reagent kit with gDNA eraser (Takara Bio, Dalian, China).

Cloning of partial coding sequences (CDSs) of *SvPCS1* and SvActin

Based on the method of Li et al. (2010), *Sophora viciifolia* cDNA acted as the template to amplify the conserved sequences of *SvPCS1* and SvActin. Two pairs of degenerate primers included *PCS1S* (5′-GAAAGGGCCTTG GAGRTGG-3′)/*PCS1A*(5′-GATATDAGCATRAAC CCYCT-3′) and *ACTS*(5′-CTCCCAGGGCTGTGTTTC CT-3′)/*ACTA*(5′-CTCCATGTCATC CCAGTTGCT-3′). Twenty-five milliliters of reaction system of PCR amplification contained 12.5 μl Premix Taq, one microliter *S. viciifolia* cDNA templates, 1 μl of each primer, and 9.5 μl RNase-Free ddH_2O. The PCR reactions were performed with a C1000 Thermal cycler (Bio-Rad, Hercules, CA, USA) through the procedure below: a 5-min denaturation at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, a 1-min annealing at 54 or 55 °C (54 °C for *SvPCS1* conserved fragment and 55 °C for SvActin conserved fragment), a 1-min extension at 72 °C, followed by a 10-min final extension at 72 °C. Subsequently, PCR products were inserted into a pGEM-T vector (Tiangen Biotech, Beijing, China) and then transformed into Escherichia coli (strain DH5α) (Tiangen Biotech, Beijing, China). Luria–Bertani (LB) medium was adopted to select the transformants. To confirm the presence of inserts, 1 μl cultured bacteria solution acted as the template DNA for PCR with primers *PCSS/PCSA* and *ACTS/ACTA*. The solutions tested to be positive were applied for sequencing (Sangon Biotech, Shanghai, China).

Analysis of gene expression

Two micrograms of RNA was exploited to synthesize the first-strand cDNA. The complementary DNA (cDNA) was reversely transcribed by applying a PrimeScript RT reagent kit with gDNA eraser (Takara Bio, Dalian, China). Specific to the qRT-PCR assay, 1 μg of total RNA was employed for the reverse-transcription, and 1 μL of the product was applied in the PCR amplification. The reaction system employed 20 μL for the qRT-PCR assay included 10 μL of SYBR Premix Ex Taq (Takara Bio, Dalian, China), and the RT-PCR was performed based on CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA). The primers for qRT-PCR here were *QSvPCS1S* (5′-TTGTTGCCAAGGAGC AGATA-3′)/*QSvPCS1A* (5′-CCTGTTTCAATACCT CTTCCTT-3′) and *QSvACTS*(5-GATGCTGAGGAT ATTCAACCC-3′)/*QSvACTA*(5′-TTTGA CCCATCCCA ACCATAA-3′) (Xu et al. 2014). The RT-PCR amplification program of *SvPCS1* and SvActin gene was initiated at 95 °C for 3 min to activate the polymerase, and then 40 cycles were performed at 95 °C for 5 s and 57 °C for 30 s. Three biological replicates were used for all genetic analyses, and relative quantification values of *SvPCS1* gene were determined based on the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen 2001). All samples were technically replicated three times. Negative controls without cDNA were run within the respective analysis. In order to verify the specificity of these amplified product after the qPCR run, the analysis of melting curve was performed under the following conditions: 95 °C for 15 s, 57 °C for 15 s, 95 °C for 10 min, and then maintained at 95 °C for 15 s.

Statistical analysis

All experimental results received a two-way analysis of variance (ANOVA) to compare As treatments and AMF inoculation as the major factors. The respective experimental treatment contained three replicates. Noticeable differences among the mentioned treatments were assessed by performing Tukey's multiple range test. The statistical analyses were conducted by using SAS Software. Figures were generated with SigmaPlot 10.0 (Systat Software Inc., San Jose, CA, USA. https://systatsoftware.com) and the package "pheatmap" in R.

Results

AMF colonization rate

The AMF colonization was found in the roots of *R. intraradices*-inoculated *S. viciifolia*, whereas it was not detected in the non-inoculated roots (Table 1). The AMF total colonization rates of *S. viciifolia* by *R. intraradices* inoculation reached 65%, 56%, and 43.5%, and arbuscules colonization rates accounted for 33%, 25% and 11.5% at 0, 50, and 100 mg kg^{-1} As levels, respectively. Notably, with the addition of As to soils, *R. intraradices* colonization was adversely affected in the *S. viciifolia* roots, which decreased with the increase in the As content in soils.

Plant growth

Plants showed the symptoms of As toxicity (e.g., leaves wilting and yellowing) when exposed to high As stress (Table 1). The shoot and root dry weight, plant height and root length of *S. viciifolia* seedlings, and the total chlorophyll content in leaves were reduced as the As content increased in soils ($P < 0.01$), whereas the shoot dry weight in *S. viciifolia* seedlings and root length in *R. intraradices*-inoculated seedlings were insignificantly different between 0 and 50 mg kg^{-1} As level.

The shoot and root dry weight, plant height and root length in *S. viciifolia* seedlings and the total chlorophyll content in leaves were evidently promoted by *R. intraradices*-inoculation ($P < 0.05$). Compared with

Table 1 Effects of *R. intraradices* on AM colonization, shoot dry weight, root dry weight, plant height, and root length of *S. viciifolia* seedlings and the total chlorophyll content in leaves at different levels of As stress

Inoculation	As (V) treatments mg kg^{-1}	AM total colonization (%)	Arbuscules colonization (%)	Shoot dry weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	Plant height (cm)	Root length (cm)	Total chlorophyll content in leaves (mg/g fw of tissue)
Non-AMF	0	0	0	1.39 ± 0.08ab	1.44 ± 0.11ab	41.40 ± 0.94ab	31.58 ± 1.16a	3.74 ± 0.06 a
	50	0	0	1.38 ± 0.07ab	1.32 ± 0.13c	39.92 ± 1.20ab	28.03 ± 1.37b	2.65 ± 0.09 c
	100	0	0	0.96 ± 0.07c	1.05 ± 0.06d	35.04 ± 1.50c	23.64 ± 1.31c	1.21 ± 0.11 e
R. intraradices	0	65.0 ± 5.50a	33.0 ± 1.50a	1.53 ± 0.10a	1.54 ± 0.12a	43.85 ± 1.37a	33.89 ± 1.56a	3.38 ± 0.05 b
	50	56.0 ± 4.00b	25.0 ± 2.50b	1.39 ± 0.06a	1.44 ± 0.11b	42.75 ± 1.45a	32.09 ± 0.93a	2.91 ± 0.07 bc
	100	43.5 ± 5.00c	11.5 ± 1.00c	1.21 ± 0.03b	1.22 ± 0.10c	38.67 ± 1.29bc	27.55 ± 1.08b	1.69 ± 0.08 d
Significance								
AMF		**	**	NS	*	*	*	*
AS				*	**	**	*	**
AMF × AS				NS	NS	NS	NS	NS

The same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test; values are means ± SD, $n = 3$; *$P < 0.05$, **$P < 0.01$, *NS* Not significant

non-inoculated *S. viciifolia*, *R. intraradices*-inoculated *S. viciifolia* seedlings achieved improvements in the shoot and root dry weight, plant height, root length, and higher total chlorophyll content in leaves, irrespective of the As treatment. Except, the root length in 0 mg kg^{-1} As level was insignificantly different between *R. intraradices*- and non-inoculated seedlings.

The As content in *S. viciifolia* shoots and roots increased, and the P content decreased with the increase in As level in soils, regardless of the *R. intraradices* inoculation ($P < 0.05$) (Table 2). The As content in *S. viciifolia* shoots and roots was obviously decreased and the P content was increased by *R. intraradices* inoculation at the identical As level in soils, except for that at 0 mg kg^{-1} As level.

Gas exchange and chlorophyll fluorescence

As stress noticeably affected the parameters of gas exchange in *S. viciifolia* plants ($P < 0.01$), as manifested by the depressed Pn, gs, and E, as well as the improved Ci (Fig. 1). However, no significant differences were identified in the Pn between 0 and 50 mg kg^{-1} As levels in *R. intraradices*-inoculated seedlings. The inoculation with *R. intraradices* in the roots of *S. viciifolia* remarkably increased Pn and gs and decreased Ci ($P < 0.01$). Nevertheless, such an inoculation insignificantly affected the E, irrespective of the *R. intraradices*-inoculation.

The Fv/Fm, ΦPSII and qP in *S. viciifolia* leaves decreased noticeably, and the NPQ significantly increased with increasing As concentration in soils ($P < 0.01$) (Fig. 2). Besides, the ΦPSII in the *R. intraradices*-inoculated

Table 2 Effects of *R. intraradices* on the As and P content in *S. viciifolia* shoots and rootsat different levels of As stress

Inoculation	As (V) treatments mg kg^{-1}	As concentration in shoots (mg/kg)	As concentration in roots (mg/kg)	P concentration in shoots (mg/g)	P concentration in roots (mg/g)
Non-AMF	0	2.55 ± 0.22e	0.24 ± 0.11e	2.35 ± 0.13b	2.84 ± 0.11b
	50	7.43 ± 0.48c	84.48 ± 6.84c	1.93 ± 0.08c	2.27 ± 0.08d
	100	13.95 ± 0.32a	176.72 ± 6.51a	1.42 ± 0.09d	1.52 ± 0.07e
R. intraradices	0	1.17 ± 0.29e	0.28 ± 0.05e	2.87 ± 0.12a	3.14 ± 0.12a
	50	5.38 ± 0.38d	66.44 ± 4.23d	2.34 ± 0.08b	2.71 ± 0.13c
	100	10.50 ± 0.97b	138.39 ± 5.78b	1.74 ± 0.11cd	2.24 ± 0.08d
Significance					
AMF		NS	NS	*	*
AS		*	*	*	*
AMF × AS		**	**	**	**

The same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test; values are means ± SD, $n = 3$; *$P < 0.05$, **$P < 0.01$, *NS* Not significant

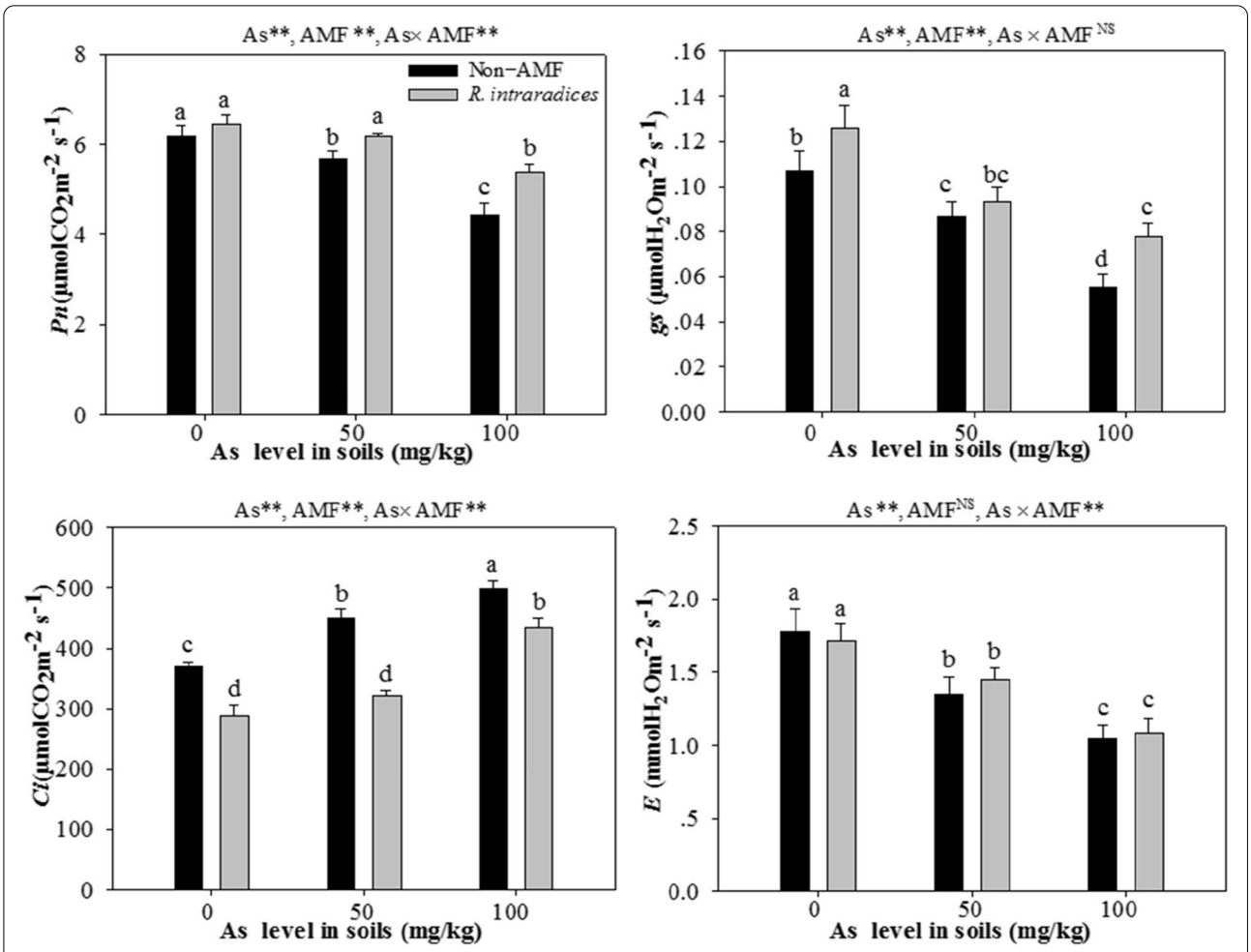

Fig. 1 Effects of *Rhizophagus intraradices* on net photosynthetic rate (*Pn*), stomatal conductance (g_s), intercellular CO_2 concentration (*Ci*), and transpiration rate (*E*) in *Sophora davidii* leaves at different levels of As stress. Note: the same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test; values are means ± SD, $n = 3$; *$P < 0.05$, **$P < 0.01$, NS not significant

seedlings showed an apparent difference between 0 and 50 mg kg^{-1} As levels. The inoculation with *R. intraradices* significantly increased the Fv/Fm, ΦPSII and qP, and decreased NPQ, as compared with non-inoculated plants at the identical As level ($P < 0.01$).

Oxidative damage

The oxidative damage in *S. viciifolia* leaves and roots was aggravated with the increase of As concentration in soils (Fig. 3). With the increase in the As content in soils, MDA and $O_2^{\bullet-}$ content in the leaves and roots increased distinctly. H_2O_2 content in leaves were insignificantly different between 0 and 50 mg kg^{-1} As treatments. Still, the H_2O_2 content was peaked at 100 mg kg^{-1} As under *R. intraradices*- and non-inoculation conditions.

The inoculation with *R. intraradices* resulted in significantly less oxidative damage in *S. viciifolia* leaves and roots ($P < 0.01$). At 50 and 100 mg kg^{-1} As level in soils, the MDA, H_2O_2, and $O_2^{\bullet-}$ content in leaves and roots decreased significantly through the *R. intraradices*-inoculation, except for the H_2O_2 content in leaves at 50 mg kg^{-1} As level. At 0 mg kg^{-1} As level, no difference was identified in the MDA, H_2O_2, and $O_2^{\bullet-}$ content in leaves and roots between *R. intraradices*- and non-inoculation treatments, whereas the MDA content in the roots decreased through the *R. intraradices*-inoculation.

Antioxidant enzyme activities

Regardless of whether *S. viciifolia* was inoculated with *R. intraradices*, the SOD, POD and CAT enzyme activities in *S. viciifolia* leaves and roots increased with the elevation of the As level ($P < 0.01$), and they were peaked at 100 mg kg^{-1} As (Fig. 4). The inoculation with *R. intraradices* evidently improved the SOD, POD, and CAT enzyme activities in leaves and roots

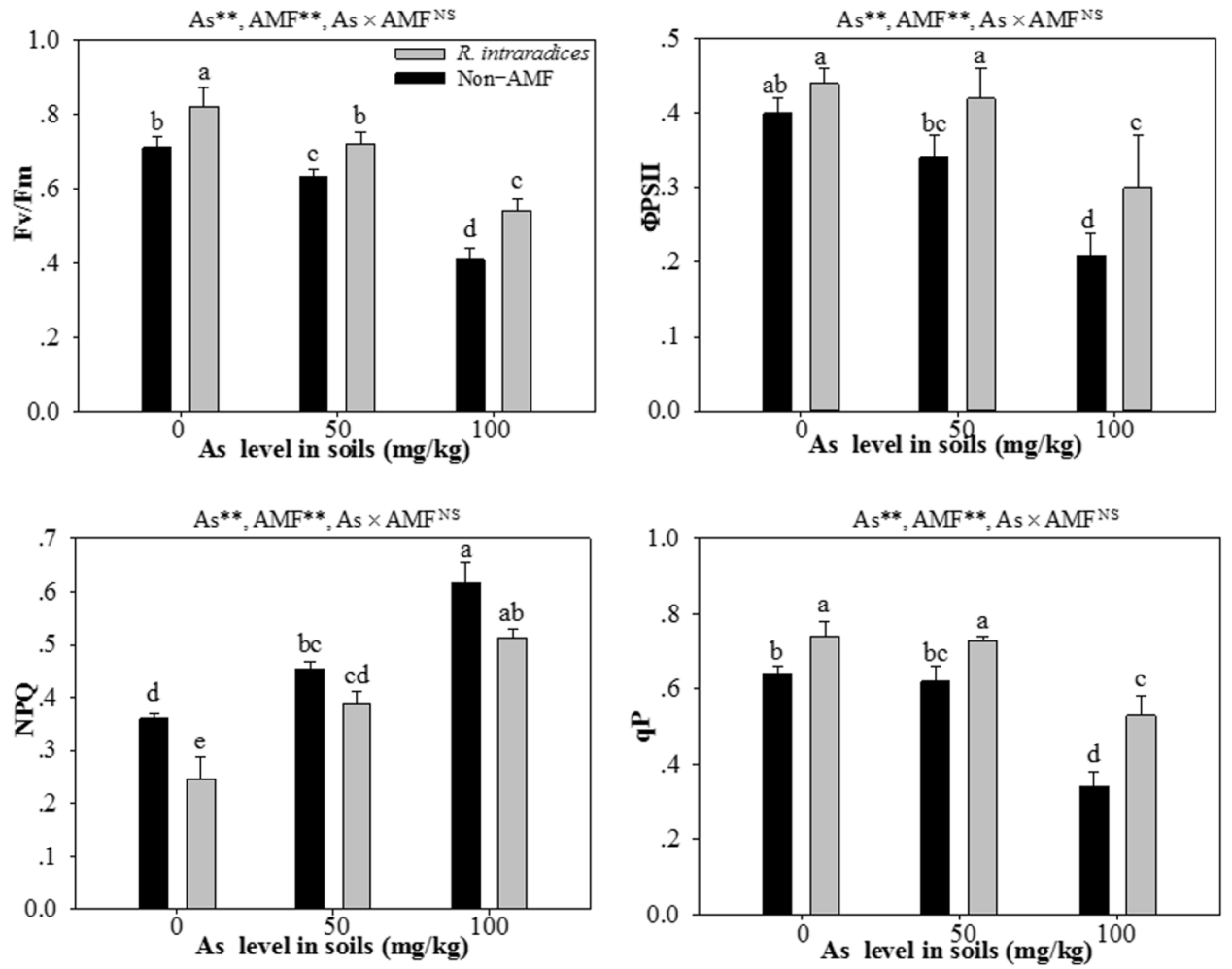

Fig. 2 Effects of *Rhizophagus intraradices* on maximum quantum yield in the dark-adapted state (*Fv/Fm*), actual quantum yield in the light-adapted steady state (*ΦPSII*), nonphotochemical quenching values (*NPQ*), and photochemical quenching values (*qP*) in *Sophora davidii* leaves at different levels of As stress. Note: the same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test; values are means ± SD, $n = 3$; *$P < 0.05$, **$P < 0.01$, NS not significant

at 50 and 100 mg kg^{-1} As level ($P < 0.01$). However, at 0 mg kg^{-1} As level, only the enzymatic activities of SOD and POD in *S. viciifolia* leaves were remarkably improved through the *R. intraradices* inoculation.

The gene expression of svpcs1

The *R. intraradices* inoculation noticeably affected the gene expression of *SvPCS1* in *S. viciifolia* seedlings ($P < 0.01$) (Fig. 5). At the identical As level, the gene expression of *SvPCS1* in the leaves and roots was distinctly up-regulated through the *R. intraradices*-inoculation. The As content in soils significantly affected the gene expression of *SvPCS1* in *S. viciifolia* seedlings ($P < 0.01$), which was manifested as the gene expression of *SvPCS1* in the leaves and roots was evidently up-regulated with the elevated As level in soils. In non-mycorrhizal plants, the gene expression of *SvPCS1* in the leaves and roots in 100 mg kg^{-1} As levels was up-regulated through the *R. intraradices*-inoculation, whereas no obvious difference was reported between 0 and 50 mg kg^{-1} As levels. In the *R. intraradices*-inoculated *S. viciifolia* seedlings, the gene expression of *SvPCS1* in the leaves and roots was highest in the 100 mg kg^{-1} As levels, and lowest in the 0 mg kg^{-1} As levels. Furthermore, the expression level of SvPCS1 gene in the *R. intraradices*-inoculated roots was significantly higher than that in the leaves at the same As level treatment ($P < 0.01$).

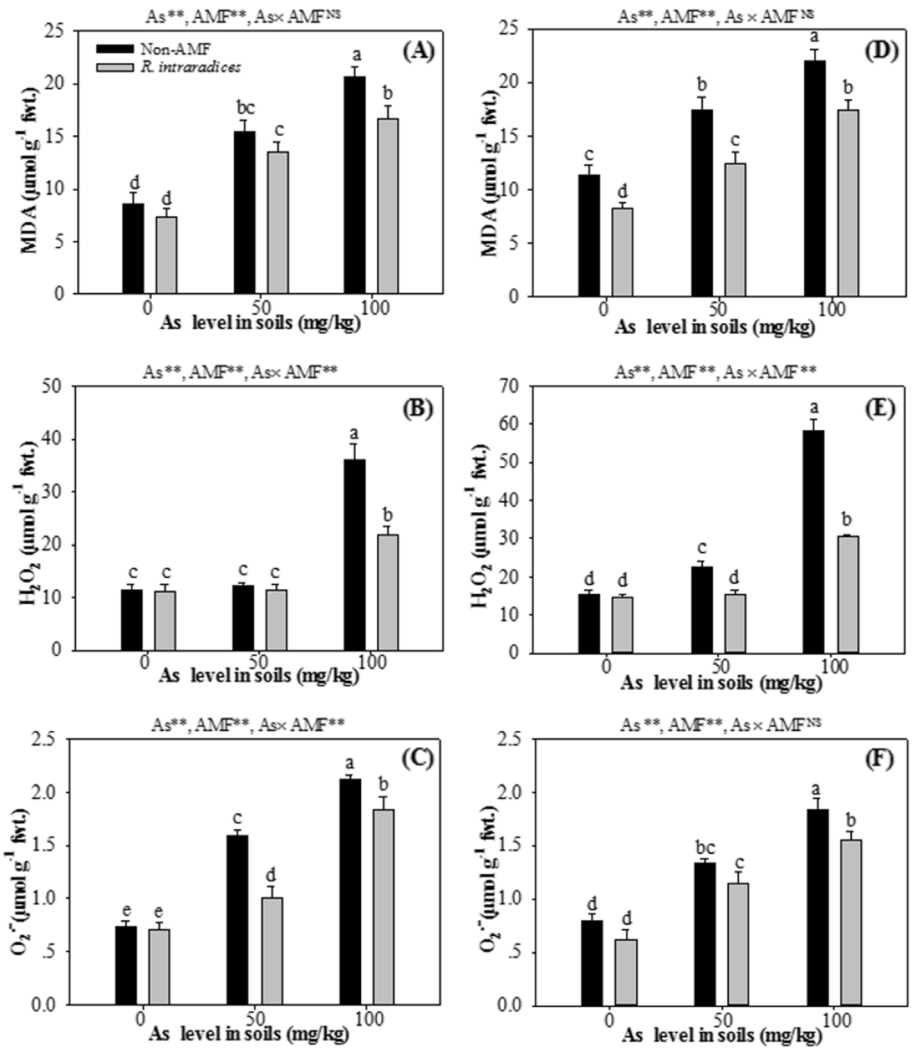

Fig 3 Effects of *Rhizophagus intraradices* on reactive oxygen species in *Sophora davidii* leaves (**A**, **B**, **C**) and roots (**D**, **E**, **F**) at different levels of As stress. Note: MDA: malondialdehyde, H_2O_2: hydrogen peroxide, $O_2^{\bullet-}$: superoxide anion. The same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test; Values are means ± SD, $n = 3$; $^*P < 0.05$, $^{**}P < 0.01$, NS not significant

Discussion

The AMF colonization rate in *S. viciifolia* roots decreased with the elevation of As content in soil in our study. Similar As negative effects on AMF colonization were previously found in other host plants, such as *Glycine max* L. (Spagnoletti and Lavado 2015), *Lolium perenne* L. (Dong et al. 2008), and *Helianthus annuus* L. (Ultra et al. 2007). However, some studies reported no decrease in the AMF colonization rate (Christophersen et al. 2009) and also an increase (Al Agely et al. 2005) when the As solution was artificially introduced into soils. Despite such a difference of AMF colonization rate to As stress, each AMF symbiosis could contribute to host plants (Sharma et al. 2017). Our results indicated that As negatively affected the AMF colonization in the rhizosphere soil of *S. viciifolia* roots. When exposed to As toxicity, plants produced more root exudates, thus the plant supply of carbonaceous compounds to AMF was reduced, which hampered the AMF pre-symbiotic process, such as decrease in spore germination and hyphal growth in plant rhizosphere (Spagnoletti et al. 2017; Spagnoletti and Lavado 2015).

The efficiency of AMF-inoculation for plant against HM stress could be visually indicated by plant biomass (Chen et al. 2015). High As levels in soils often jeopardized the normal plant growth, and some toxicity symptoms (e.g., biomass decrease, stagnation in plant growth, wilting, and necrosis of leaf blades) could be shown (De Andrade et al. 2015; Srivastava and Sharma 2013). In this

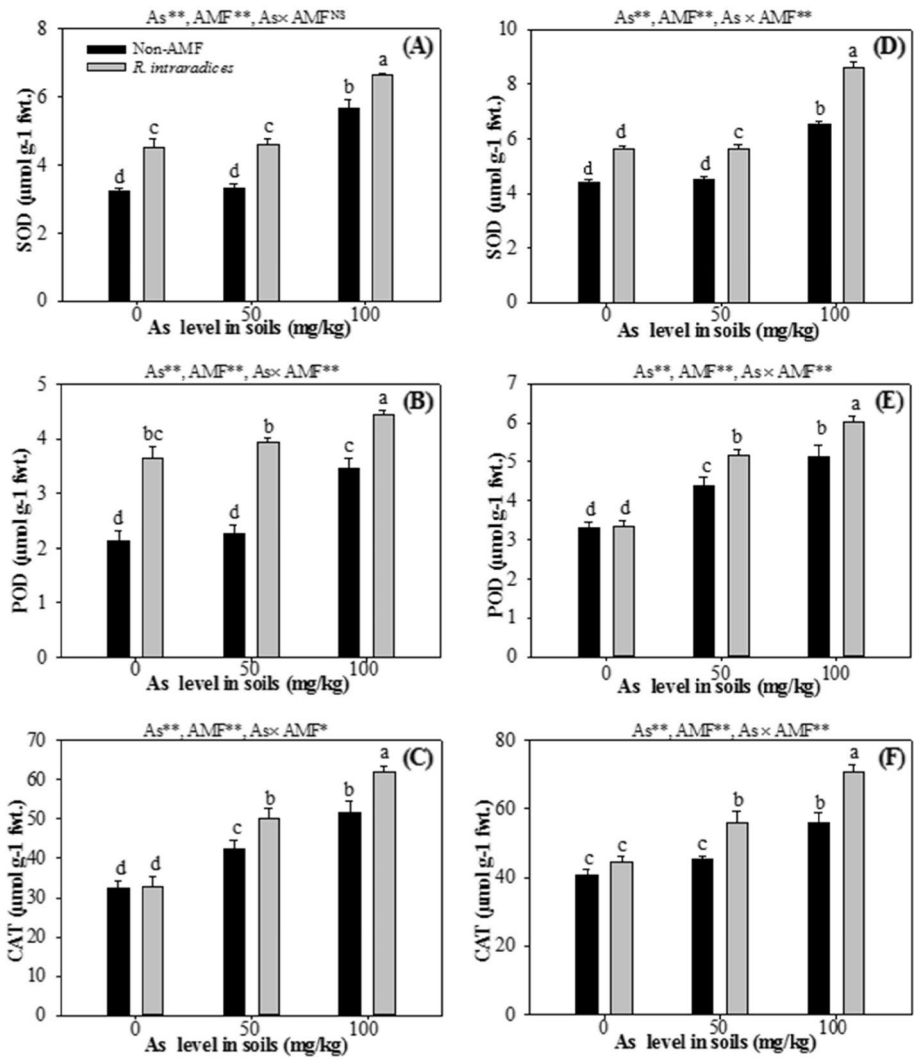

Fig. 4 Effects of *Rhizophagus intraradices* on antioxidant enzyme in *Sophora davidii* leaves (**A**–**C**) and roots (**D**–**F**) at different levels of As stress. Note: SOD: superoxide dismutase, POD: peroxidase, CAT: catalase. The same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test; values are means ± SD, $n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$, NS not significant

study, the growth parameters of *S. viciifolia* seedlings were reduced with the increase of As content in soils. But the *R. intraradices*-inoculated *S. viciifolia* grew better than non-inoculated seedlings at all As levels, which suggested that the *R. intraradices* symbiosis could play a positive role on mitigating As stress in *S. viciifolia* seedlings. This result was consistent with other previous studies which showed biomass losses in plants by high As toxicity and also proved the role of AMF in improving the As tolerance by increasing plant growth (Christophersen et al. 2009; Das et al. 2017a, b; De Andrade et al. 2015). The improved parameters of plant growth under As stress were closed due to improved mineral nutrition via AMF extraradical hyphal networks (Ahmed et al. 2006; Garg and Chandel 2011).

As and P belonged to the same family in the periodic table of chemical element, both of them had the similar chemical property in nature, and competed for the same transport systems on plant cell membrane (Li et al. 2019). In the present study, the As contents in the roots and shoots of *R. intraradices*-inoculated *S. viciifolia* were significantly lower, and the P content were higher than those of non-inoculated seedlings. AMF-inoculated plants kept a higher P:As ratio, consequently prevented replacement of P by As in the process of photophosphorylation for the synthesis of ATP, ultimately As-induced negative impact

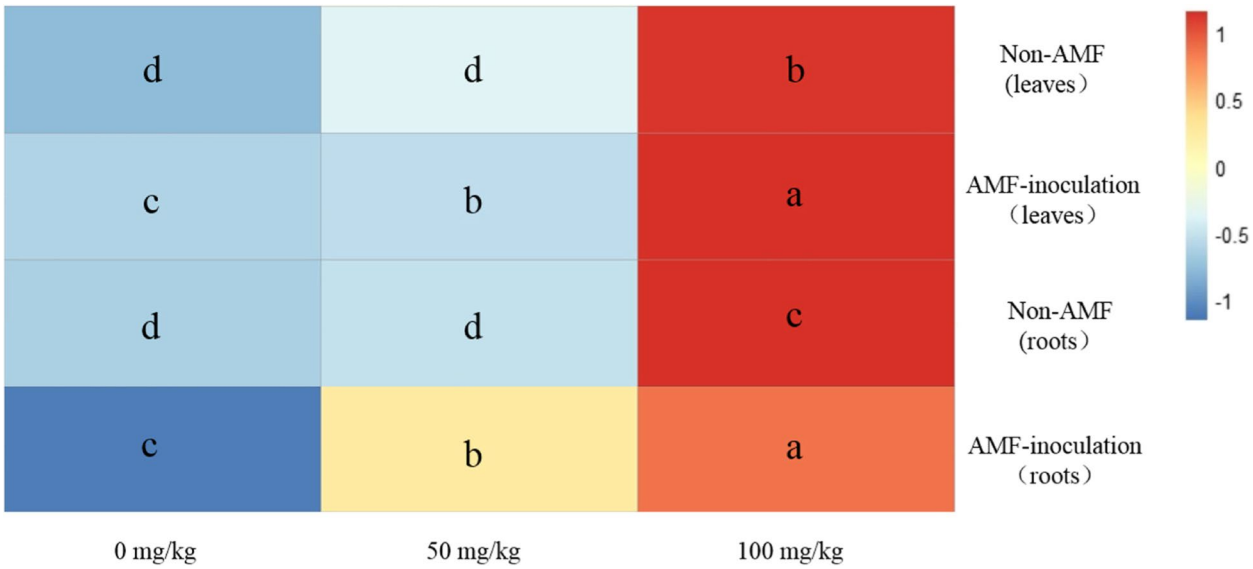

Fig. 5 Effects of *Rhizophagus intraradices* on expression of *SvPCS1* in roots and leaves of *Sophora davidii*. at different levels of As stress. Note: Different letters within each gene indicate significant differences ($P < 0.05$) using Tukey's test; $n = 3$

on photosynthesis were alleviated in AMF-inoculated plants (Sharma et al. 2017). Our results also found that the shoot and root dry weight, plant height and root length in the *R. intraradices*-inoculated *S. viciifolia* seedlings were distinctly improved as compared with those in the non-inoculated seedlings under As stress. Our results suggested that *R. intraradices* symbiosis increased P absorption, reduced As content in plant tissues and promoted the growth of host plants, which were concordant with other reports (Gonzalez-Chavez et al. 2002; Sharples et al. 2000; Zhang et al. 2020). The lower As content and the greater biomass of the AMF-inoculated seedlings in this study verified the previously mentioned mechanism of "growth dilution effect" (Chen et al. 2015; Li et al. 2018; Spagnoletti and Lavado 2015).

To avoid oxidative damages, plants were equipped with antioxidant enzymes system that could cope with the accumulation of ROS in plants. *Funneliformis* mosseae and *Diversispora spurcum* inoculation decreased the H_2O_2 and MDA contents and enhanced SOD and CAT) activities in maize leaves under multiple HM (Pb, Zn, Cd, and As) stress (Zhan et al. 2018). Similarly, *R. intraradices* and *Glomus etunicatum* inoculation were reported to improving the As tolerance by increasing the antioxidant enzyme activity and antioxidant content in wheat (Sharma et al. 2017). In this study, *R. intraradices* inoculated *S. viciifolia* displayed the lower H_2O_2, $O_2^{\bullet -}$, and MDA levels, and showed the higher antioxidant activities of SOD, CAT and POD than the non-inoculated seedlings under As stress. Enhanced activities of antioxidant enzymes may be due to higher uptake of these micronutrients and lower cellular As distribution in AMF-inoculated plants (Spagnoletti et al. 2016). Thus, a decrease in the oxidative damage and an improvement in antioxidant enzymes could act as the vital strategies by which AMF protect host plants against As stress.

AMF symbiosis enhanced the photosynthesis of host plants under stress conditions, due to higher gas exchange rates and efficiencies by means of mycelia growth and respiration (Kaschuk et al. 2009). In this study, the inoculation with *R. intraradices* increased the *Pn*, *gs* and *E*, and such an inoculation also decreased *Ci* in *S. viciifolia* leaves under As stress, which indicated that *R. intraradices* inoculation improved the photosynthesis of *S. viciifolia* under As stress. The Pn increase through the AMF inoculation was related to a higher *gs* in AMF-inoculated seedlings than that in non-inoculated plants (Chen et al. 2015). Other reports also showed that the *R. irregularis* inoculation tended to increase the E and gs in rice leaves under As exposure (De Andrade et al. 2015).

The effects of *R. intraradices* inoculation on the photochemical capacities of *S. viciifolia* leaves under As stress were assessed by using a chlorophyll fluorescence analysis. The Fv/Fo value, representing the photochemical capacity of PSII in plants, indicated the quantity and size of the active photosynthetic centers in the chloroplasts (Xu et al. 2014). In the present study, the Fv/Fm exceeded 0.8 in the *R. intraradices*-inoculated seedlings without As stress, and it was still lower than 0.8 in all of the plants under As stress, which indicated that As

stress caused the chronic photoinhibition and impaired the photochemical activity in *S. viciifolia* leaves. The PSII and qP in *S. viciifolia* leaves decreased with the increase in As content, but both were higher in *R. intraradices*-inoculated seedlings than in non-inoculated seedlings, which were related to down-regulate the electron transport rate of PSII by As toxicity and to elevate the electron transport rate by AMF symbiosis (De Andrade et al. 2015). It was reported that *R. intraradices* improved the efficiency of PSII photochemistry and reduced the adverse effect of Pb stress on photosynthesis in *S. viciifolia* (Xu et al. 2014). The increased Fv/Fm and PSII in AMF-inoculated *S. viciifolia* suggested an improved efficiency of PSII photochemistry, compared with that of non-inoculated *S. viciifolia*, other studies identified the similar results, which showed that AMF symbiosis improved the performance of photosynthesis-related processes in rice under As stress (De Andrade et al. 2015). In our study, the NPQ significantly increased with the improvement of the As level in soils, but decreased by *R. intraradices*-inoculation. It indicated AMF symbiosis alleviated the photoinhibition and possibly restored oxidative damages to the chloroplasts. The improved photosynthetic capacity in AMF-inoculated maize under salt stress were observed, due to higher gas exchange and photochemical and non-photochemical efficiencies (Sheng et al. 2008). In our study, AMF symbiosis enhanced photosynthesis of *S. viciifolia* seedlings under As stress by improving the efficiency of PSII photochemistry to use light energy and transport the electron.

PCs played a pivotal role in the homeostasis, the immobilization and the transportation for some HMs (e.g., Cu, Pb, and Zn) (Xu et al. 2014). The elevated HM content and the present GSH in the cytoplasm of plants activated the *PCS* enzyme, on that basis, the PCs biosynthesis was initiated (Xu et al. 2014). The expressions level of plant *PCs* genes were also proved to be regulated under some HM stresses, but the expression patterns were still controversial. The gene expression of *SvPCS1* in *S. viciifolia* leaves and roots was evidently up-regulated with the elevated As level in soils in this study. The regulating expression of *PCS* gene in plants complied with the requirement of resilient defense against different HMs (De Andrade et al. 2015). The gene expression of tobacco phytochelatin synthase (*NtPCS1*) exhibited the increased tolerance to As stress (Lee and Hwang 2015). The exposure to high As stress induced the overexpression of *PCS* catalyzing the formation of PCs, while the PCs-As complex alleviated the stress by chelating and transferring As ions from the cytoplasm into vacuole (De Andrade et al. 2015).

In this study, *SvPCS1* expressed both in non- and *R. intraradices*-inoculated *S. viciifolia* seedlings, and *R. intraradices*-inoculation noticeably up-regulated the expression of *SvPCS1*, which was attributed to the lower As toxicity in the cytoplasm of *R. intraradices*-inoculated root cells, compared with non-inoculated ones (Xu et al. 2014). Christophersen et al. (2012) also found that both *R. intraradices* and *F. mosseae* inoculated *Medicago truncatula*. had higher expressions of *MtPCS* than non-mycorrhizal plants under As stress.

Conclusion

In this study, excessive As in soils exerted the multiple negative effects on various physiological and biochemical parameters of *S. viciifolia* seedlings. However, *R. intraradices* symbiosis improved the plant biomass, gas exchange, chlorophyll fluorescence, activities of antioxidant enzymes, and upregulated the expression of *SvPCS1*in *S. viciifolia* seedlings, which suggested that the significant role of *R. intraradices* in improving the growth performance of *S. viciifolia* seedlings under As stress. Thus, this study demonstrated AMF inoculation was a feasible way to improve the As tolerance on woody leguminous species. The inoculation with AMF in woody leguminous may be a viable technology in phytoremediation and land reclamation in As-contaminated areas.

Abbreviations

AMF: Arbuscular mycorrhizal fungi; As: Arsenic; *Pn*: The photosynthetic rate; *gs*: The stomatal conductance; *E*: The transpiration rate; *Fv/Fm*: The maximal photochemical efficiency of PSII photochemistry; *ΦPSII*: The actual quantum yield; *qP*: The photochemical quenching values; MDA: Malondialdehyde; $O_2^{\cdot-}$: Superoxide radical; ROS: Reactive oxygen species; H_2O_2: Hydrogen peroxide; HMs: Heavy metals; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase; PSII: Photosystem II; PCs: Phytochelatins; GSH: Tri-peptide glutathione; K: Potassium; P: Phosphorus; N: Nitrogen; Cd: Cadmium; GiPT: High-affinity Pi/As transporter; Fs: The steady-state fluorescence; Fm′: The maximal fluorescence; Fo′: The minimal fluorescence level in the light-adapted state; (Fm-Fo)/Fm: The maximum quantum yield of the PSII photochemistry; (Fm′-Fs)/Fm′: The actual quantum yield of PSII electron transport; NPQ: The quenching due to non-photochemical dissipation; qP: The coefficient of photochemical quenching; CDSs: Cloning of partial coding sequences; PCR: Polymerase chain reaction; ANOVA: Analysis of variance; PCS: Phytochelatin synthase.

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Authors' contributions

Minggui Gong designed research and experiments. Qiaoming Zhang and Yanan Wei wrote the main manuscript text. Jiangfeng yuan and Shanshan Xu performed the plant pot experiment. Qingshan Chang provided critical reading and revising suggestion. All authors read, reviewed, and approved the manuscript.

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Declarations

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N/A

Consent for publication
All the authors have approved the manuscript that is enclosed.

Competing interests
The authors declare that they have no competing interests.

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