https://doi.org/10.17221/520/2020-PSE

Mycorrhizal fungi enhance flooding tolerance of peach through inducing proline accumulation and improving root architecture

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Citation: Zheng F.-L., Liang S.-M., Chu X.-N., Yang Y.-L., Wu Q.-S. (2020): Mycorrhizal fungi enhance flooding tolerance of peach through inducing proline accumulation and improving root architecture. Plant Soil Environ., 66: 624–631.

Abstract: This study aimed to evaluate the effect of an arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* on plant growth, root architecture, and proline metabolism in roots of peach (*Prunes persica* L.) under non-flooding and flooding conditions. The 12-day flooding dramatically inhibited root colonisation of *G. mosseae*, but induced a large number of extraradical mycelia. Although the flooding treatment also relatively inhibited growth and root architecture of peach, the mycorrhizal fungal inoculation dramatically increased shoot and root biomass, plant height, stem diameter, number of 1st- and 2nd-order lateral roots, root total length (mainly 0–1 cm and > 3 cm long), root surface area, and root volume under flooding. The study also revealed distinctly higher proline accumulation in the roots of mycorrhizal plants than non-mycorrhizal plants under both non-flooding and flooding conditions, accompanied by higher Δ^1-pyrroline-5-carboxylate synthase (P5CS) activity and lower δ-ornithine transaminase and proline dehydrogenase activities. In addition, the *PpP5CS1* gene expression was up-regulated by flooding and mycorrhization. This study concluded that mycorrhizal fungi enhanced flooding tolerance of peach through inducing proline accumulation and improving root architecture.

Keywords: fruit tree; osmotic adjustment; symbiosis; water stress

Flooding, a kind of abiotic stress, causes serious harm to plant growth and development by interfering a series of physiological and biochemical activities of plants, finally resulting in the death of the plant. Under flooded conditions, the roots of plants completely soak in the soil of saturated water, inducing low oxygen or the anaerobic environment in the rhizosphere, leaching of mineral elements, and anaerobic respiration, which is not good for the plant growth (Lin et al. 2020). Peach is a widely cultivated, deciduous fruit tree in the world. Peach is characterised by shallow roots and high root respiration, accompanied by a high demand for oxygen and poor flooding tolerance. In peach orchards, high rainfall, improper irrigation, and poor drainage often lead to flooding in the orchard (Xiao 2015). Therefore, it is urgent to increase the flooding tolerance of peach trees.

Arbuscular mycorrhizal fungi (AMF), a class of beneficial soil microorganisms, can form mutual symbiont with more than 80% of land plants *viz*. arbuscular mycorrhiza (Wu et al. 2013a, Xie et al. 2020, Zhang et al. 2020). It is known that AMF can improve the stress resistance of host plants, including salinity, drought, heavy metals, and other adverse environments (Begum et al. 2019, He et al. 2019, Wu et al. 2019b, Zou et al. 2020). Furthermore, AMF-released

Supported by the Open Fund of Engineering Research Center of Ecology and Agricultural Use of Wetland, Ministry of Education, Project No. KFT202005; by the Innovation Training Program for Students of Yangtze University, Project No. Yz2020363, and by the Key Cultivation Project in college students' extracurricular academic science and technology works competition.

https://doi.org/10.17221/520/2020-PSE

glomalin also cements soil particles, stabilises soil aggregation, and contributes soil nutrients (He et al. 2020, Meng et al. 2020). The AMF-produced glomalin plays an important role in adversity by chelating heavy metals in the soil and retaining soil moisture and thus is considered as a potential soil conditioner (Zou et al. 2016, Chi et al. 2018, Lü et al. 2019). It is reported that the rhizosphere of wetland plants inhabits various AMF species (Wirsel 2004). Wu et al. (2013b) reported that *Diversispora spurca* could improve the growth performance and root morphology of citrus under flooding stress, enhance the activity of antioxidant enzymes, and thus improve the flooding tolerance of citrus. Similarly, Zou et al. (2014) also found greater catalase and superoxide dismutase activities in trifoliate orange after inoculation with *D. spurca* under 36-day flooding stress conditions. In *Casuarina equisetifolia* plants, *Glomus clarum* induced lower ethanol accumulation under flooding stress, compared with non-mycorrhizal treatment (Osundina 1998). These results fully demonstrate the positive effect of AMF in enhancing the flooding tolerance of host plants. However, there are limited reports on the effects of AMF on proline metabolism in plants under flooding stress.

Proline is not only used as an osmotic substance to regulate osmotic balance but also eliminates free radicals and protects the integrity of cell structure. Hence, the accumulation of proline is closely linked to the tolerance of plants to environmental stress (Quan et al. 2007). There are two proline synthesis pathways in plants: glutamate synthesis pathway and ornithine synthesis pathway, the key enzymes of which are Δ^1-pyrroline-5-carboxylate synthase (P5CS) and δ-ornithine transaminase (δ-OAT) (Chun et al. 2018). Proline degradation is a reversal of the glutamate synthesis pathway, and the key enzyme in this process is proline dehydrogenase (ProDH). Under non-stress conditions, proline synthesis and degradation in plants are in equilibrium; under stress conditions, plants can promote proline accumulation by regulating proline synthesis and decomposition so as to resist the harm of adversity. Studies have demonstrated that the accumulation, synthesis, and decomposition of proline in plants could be regulated by AMF to respond to salt stress and drought stress (Chun et al. 2018). Increasing or decreasing proline levels of host plants have been reported after AMF inoculation, indicating the complex network of proline metabolism in mycorrhizal plants (Abbaspour et al. 2012, Garg and Baher 2013, Guo et al. 2015, Wu et al. 2017). Therefore, more studies focusing on proline metabolism under abiotic stress can illustrate the role of mycorrhizal fungi.

In this study, we aimed to evaluate the effect of AMF on plant growth, root architecture, and proline metabolism in roots of peach (*Prunes persica* L.) under non-flooding and flooding conditions.

MATERIAL AND METHODS

Mycorrhizal fungal inoculants. An arbuscular mycorrhizal fungus, *Glomus mosseae* (T. H. Nicolson & Gerd.) Gerd. & Trappe was selected. The fungal strain was identified from the rhizosphere of *Incarvillea younghusbandii* in Dangxiong, China, and provided by the Bank of Glomeromycota in China. In our earlier studies of three AMF species (*Glomus mosseae*, *G. versiforme*, and *Paraglomus occultum*), *Glomus mosseae* had shown the superior capacity for improved growth and nutrient acquisition of peach (Wu et al. 2011). After isolated and identified, spores were propagated through potted white clover for three months. Subsequently, the aboveground parts of white clover were removed, and the roots and growth substrate were collected as mycorrhizal fungal inoculants, in which spores (15 spores/g), mycorrhizal hyphae, and AMF-infected root segments were contained.

Plant culture. The peach seedlings of six-leaf age with the same size and non-mycorrhization were transplanted to a 2.76-L plastic pot, where 2.3 kg of autoclaved (121 °C, 0.11 Mpa, 2 h) soils were supplied. The physical and chemical properties of the soil were pH 6.2, 9.4 g/kg soil organic carbon, 120.3 mg/kg available nitrogen, 16.2 mg/kg Olsen-P, and 22.7 mg/kg available potassium. When the peach seedlings were transplanted, AMF inoculation was conducted. Each AMF-inoculated seedling received 30 g of the mycorrhizal fungi inoculums; for the non-AMF-inoculated seedling, autoclaved 30 g of mycorrhizal inoculums was applied.

After 66 days of mycorrhizal treatments, water treatments began. Flooded pots were put into a 7.7-L plastic bucket, and tap water was filled into the bucket to make the water level to 3 cm above the pot. Non-flooded pots were also put into the plastic bucket without tap water. When the water level in the plastic bucket was less than 3 cm above the pot, the lost water was replenished.

Experimental design. A 2 × 2 two-factor experimental design was conducted in this experiment. Factor 1 was AM fungal inoculations with and with-

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out *G. mosseae*, and factor 2 was water treatments, including flooding and non-flooding. A total of four treatments, including non-flooding + AMF, non-flooding – AMF, flooding + AMF, and flooding – AMF, were arranged, and each treatment replicated three times, having a total of 12 pots.

Determination of root mycorrhizal colonisation and root morphology. After 12 days of flooding, plants were harvested and divided into the shoot and the root. Then, the root segments with 1–2 cm long were cut, cleared with 10% (*w*/*v*) KOH solutions, and stained with 0.05% (*w*/*v*) trypan blue in lactic acid phenol (Phillips and Hayman 1970). Root mycorrhizal colonisation degree was estimated as the percentage of the AMF-infected root segment length and the observed total root length.

The complete root system was scanned using the Epson Perfection V700 Photo Dual Lens System (J221A, Jakarta Selatan, Indonesia) to obtain the root graphics, and then the root morphological parameters, including total length, different lengths of roots, projected area, surface area, volume, and average diameter were analysed using a WinRHIZO software (Regent Instruments Inc., Quebec, Canada) for the scanned root graphics.

Determination of proline, proline synthetases, and degrading enzyme in roots. Proline content of roots was extracted with 0.3 g root samples and 3% sulfosalicylic acid solution at 100 °C for 10 min and then filtered (Bates et al. 1973). The 1 mL filtrate was mixed with 1 mL glacial acetic acid and 1 mL acid-ninhydrine reagent at 100 °C for 30 min, and then 2 mL toluene (chromatographic grade) was added after cooling. The upper liquid was shaken well and centrifuged at 3 000 *g* for 5 min, and the coloration was determined at 520 nm.

Proline synthetases, including P5CS and δ-OAT activities, were assayed according to the protocols described by Zou et al. (2013). Among them, root P5CS was extracted with 0.5 mol/L Tris-HCl buffers (pH 7.5) containing 10 mmol/L $MgCl_2$, 2 mmol/L phenylsulfonyl fluoride, and 2% polyvinylpyrrolidone; δ-OAT was extracted with 50 mmol/L phosphate buffers (pH 8.0) containing 1 mmol/L dithiothreitol.

ProDH, a kind of proline degrading enzyme, was determined by Zhao et al. (2002). A 0.25 g root sample was homogenised with 6 mol/L phosphate buffers (pH 7.8) containing 1 mmol/L EDTA. The homogenate was centrifuged at 4 000 × *g* for 15 min. The supernatant was added with Triton X-100 (10 μL) to a final concentration of 0.15%, vortexed and placed in an ice bath for 30 min, and then centrifuged at 20 000 × *g* for 20 min. The volume of the reaction mixture was 2.5 mL, containing 1.6 mL 0.15 mol/L bicarbonate buffer (pH 10.3), 0.2 mL 0.1 mol/L L-proline, 0.2 mL 0.9 mmol/L 2,6-dichlorophenol indophenol, and 0.5 mL supernatant, which was incubated at 30 °C for 5 min. A 0.2 mL phenazine methyl sulfate reagent (9 mg/mL, ready-to-use) was added, and the absorbance was detected at 600 nm. One enzyme activity unit (U) was defined as the change in absorbance at 600 nm for one minute.

Relative expression of the *PpP5CS1* gene in roots. The total RNA in peach roots was extracted from 0.2 g fresh samples with an EASY spin plus Plant RNA mini kit (RN38, Aidlab Biotechnologies Co., Ltd, Beijing, China), and reverse transcription was performed using a TRUEscript 1st Strand cDNA Synthesis Kit with gDNA Eraser (PC5402, Aidlab Biotechnologies Co., Ltd, Beijing, China). Based on the study of Gao et al. (2019), specific primers of *PpP5CS1* were as follows: AAGCAGATAATTTCAGGACTCCA (*PpP5CS1-F*) (5'→3'); GCAGTCACATCATCAACAATTTC (*PpP5CS1-R*) (5'→3'). The qRT-PCR system was as follows: 10 μL SYBRGREEN PCR Master Mix, 6.4 μL ddH_2O, 2 μL cDNA, and 0.8 μL of each primer (forward and reverse). qRT-PCR was performed in the Bio-Rad CFX connect-time system under the following conditions: 95 °C for 30 s, 40 cycles, 95 °C for 5 s, 60 °C for 10 s, and 72 °C for 30 s. According to Kenneth and Schmittgen (2001), the relative expression of genes was calculated by the $2^{-\Delta\Delta Ct}$ method, based on translation elongation factor 2 as the house-keeping gene. The measured transcripts were normalised to the relative expression value in non-AMF-inoculated plants under non-flooding conditions.

Statistical analysis. The experimental data were analysed by the two-way analysis of variance (ANOVA) conducted by SAS (8.1) software (Cary, USA) and the least significant difference test was used to compare the significant difference among treatments at the 5% level.

RESULTS AND DISCUSSION

Changes in root mycorrhizal colonisation in response to flooding. No mycorrhizal fungal colonisation was found in the root of the non-AMF-inoculated peach seedlings, and AMF-inoculated peach seedlings represented 36.80% to 48.20% of root mycorrhizal colonisation degree (Table 1). And, 12-day flooding stress significantly reduced root mycorrhizal colonisation by 23.65%, compared with non-flooding

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Table 1. Effects of arbuscular mycorrhizal fungus (AMF) (*Glomus mosseae*) on plant growth and mycorrhizal colonisation of peach seedlings grown in non-flooding and flooding

Water treatment	AMF treatment	Root mycorrhizal colonisation (%)	Shoot biomass (g FW/plant)	Root biomass (g FW/plant)	Plant height (cm)	Stem diameter (mm)
Non-flooding	+AMF	48.20 ± 3.53^{a}	12.8 ± 0.7^{a}	3.7 ± 0.3^{a}	69.2 ± 5.5^{a}	3.07 ± 0.15^{a}
	–AMF	0.00 ± 0.00^{c}	10.7 ± 1.0^{b}	3.0 ± 0.1^{b}	60.5 ± 3.4^{b}	2.23 ± 0.15^{b}
Flooding	+AMF	36.80 ± 0.90^{b}	6.6 ± 1.2^{c}	2.9 ± 0.1^{b}	59.1 ± 3.5^{b}	1.70 ± 0.10^{c}
	–AMF	0.00 ± 0.00^{c}	4.5 ± 0.9^{d}	2.5 ± 0.2^{c}	46.7 ± 1.4^{c}	1.40 ± 0.10^{d}
Significance						
Flooding		**	**	**	**	**
AMF		**	**	**	**	**
Flooding × AMF		**	ns	ns	ns	**

Data (means ± standard deviation, n = 3) followed by different letters in the column indicate significant differences ($P < 0.05$) between treatments. **$P < 0.01$; ns – not significant; FW – fresh weight

stress. This is in agreement with Wu et al. (2013b) in *Citrus junos* Siebold ex Tanaka and also indicates that the stress of flooding inhibited the development of mycorrhiza in roots. However, we also observed a large number of mycorrhizal extraradical mycelium induction under flooding, indicating that the strain of *G. mosseae* used here has certain hypoxia tolerance. Because AMF are aerobic fungi, their growth and development are completely dependent on oxygen. An important feature of flooding is the lack of oxygen at the roots. Such an anoxic environment is not conducive to the growth and colonisation of AMF in the host under flooding. However, there were also reported that spores of *Glomus* spp. were increased with prolonged flooding time in *Typha orientalis* C.Presl and *Oryza sativa* L. plants (Ma et al. 2014).

Changes in growth performance. Flooding stress significantly inhibited the growth of peach seedlings (Table 1, Figure 1). However, inoculation with AMF significantly increased the shoot biomass, root biomass, plant height, and stem diameter of peach seedlings, by 19.62, 23.33, 14.38, and 37.67%, respectively, under the condition of non-flooding, and by 46.67, 16.00, 26.55, and 21.43% under the condition of flooding (Table 1). This showed that AMF promoted the growth of peach seedlings under flooding stress. This is consistent with the results of Wu et al. (2013b) on *Citrus junos* after inoculation with *Diversispora spurca* under waterlogging conditions. Neto et al. (2006) reported that the growth improvement of mycorrhizal plants exposed to flooding might be related to the ability of AMF to increase the osmotic solute content and N uptake of host plants. Wu et al. (2019a) proposed that the improvement of AMF on the growth of wetland plants may be significant or limited, which needs to consider the characteristics of mycorrhizal symbiosis, the dependence of host plants on AMF, and the survival environmental factors.

Changes in root architecture. Flooding stress significantly inhibited the number of lateral roots at the 1st, 2nd, and 3rd order, while AMF inoculation significantly improved the number of lateral roots at all levels (Table 2). Under the condition of non-flooding, AMF inoculation significantly increased the number of the 1st- and 2nd-order lateral root by 25.00% and 24.06%, respectively; mycorrhizal

Figure 1. Plant growth responses of non-arbuscular mycorrhizal fungus- and *Glomus mosseae*-inoculated peach seedlings grown in non-flooding and flooding. The order of treatments is non-flooding-AMF, flooding + AMF, flooding-AMF, and non-flooding + AMF, respectively, from leaf to right

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Table 2. Effects of arbuscular mycorrhizal fungus (AMF) (*Glomus mosseae*) on root architecture of peach seedlings grown in non-flooding and flooding

Water treatment	AMF treatment	Number of lateral roots			Surface area (cm^2)	Volume (cm^3)	Root length (cm)				
		1st	2nd	3rd			0 < L ≤ 1	1 < L ≤ 2	2 < L ≤ 3	3 < L	total
Non-flooding	+AMF	60 ± 6^{a}	593 ± 45^{a}	632 ± 50^{a}	155 ± 6^{a}	1.77 ± 0.03^{a}	995.3 ± 56.3^{a}	48.9 ± 6.2^{a}	12.2 ± 1.1^{a}	16.2 ± 1.0^{ab}	1 072.5 ± 63.2^{a}
	−AMF	48 ± 5^{bc}	478 ± 20^{b}	604 ± 31^{ab}	131 ± 8^{b}	1.59 ± 0.08^{b}	783.5 ± 69.4^{b}	45.1 ± 4.1^{a}	10.9 ± 0.5^{a}	14.8 ± 0.7^{b}	854.3 ± 70.9^{b}
Flooding	+AMF	54 ± 5^{ab}	438 ± 41^{c}	555 ± 41^{b}	126 ± 1^{b}	1.36 ± 0.07^{c}	784.8 ± 85.2^{b}	43.3 ± 4.1^{a}	10.1 ± 0.6^{a}	17.2 ± 0.6^{a}	855.3 ± 89.2^{b}
	−AMF	41 ± 3^{c}	366 ± 33^{c}	379 ± 33^{c}	112 ± 6^{c}	1.17 ± 0.14^{d}	639.6 ± 21.0^{c}	47.0 ± 4.0^{a}	11.2 ± 0.9^{a}	14.9 ± 0.5^{b}	712.6 ± 18.9^{c}
Flooding		ns	**	**	**	**	**	ns	ns	ns	**
AMF		**	**	**	**	**	**	ns	ns	**	**
Flooding × AMF		ns	ns	*	ns	ns	ns	ns	*	ns	ns

Data (means ± standard error, n = 3) followed by different letters in the column indicate significant differences (P < 0.05) between treatments. *P < 0.05; **P < 0.01; ns – not significant

fungal treatment significantly increased the number of 1st- and 3rd-order lateral root by 31.71% and 46.44%, respectively, under the condition of flooding. Similar results were found in *C. junos* inoculated with *Diversispora spurca* under waterlogging conditions (Wu et al. 2013b).

The flooding treatment also dramatically reduced the root surface area, volume, and total root length (mainly roots of 0–1 cm length), compared with the non-flooding treatment (Table 2). On the other hand, *G. mosseae* inoculation significantly increased the root surface area, volume, and total root length under flooded and non-flooded conditions, whilst AMF mainly improved the root length of 0–1 cm long in flooded and non-flooded conditions and > 3 cm long roots under flooded conditions. In the flooding, a series of changes occur in plant morphological characteristics, such as inducing adventitious roots (Li 1993). This is because flooding increases ethylene levels in plants, which blocks the down-transmission of auxins, thus, causing it to accumulate locally in the stems close to the water surface, leading to the formation of adventitious roots and the proliferation of lenticels (Wang et al. 2013). Root architecture in lateral root number and root morphology has been considered as an important factor in enhancing tolerance of abiotic stress (Wu et al. 2013b). Great root architecture, in turn, absorbs more water and mineral nutrients. Therefore, flooded mycorrhizal plants showed a better root architecture, which is essential for mycorrhizal plants to resist flooding.

Changes in root proline accumulation. The increase of proline content is one of the self-defense responses of plants under adversity conditions (Wang et al. 2013, Garg and Baher 2013, Chun et al. 2018). Figure 2 showed that the flooding treatment strongly promoted the accumulation of proline in roots, which increased by 64.63% in the mycorrhizal plants and 45.45% in the non-mycorrhizal plants, indicating that the mycorrhizal plants could accumulate more proline under flooded conditions. On the other hand, mycorrhizal plants showed 48.48% and 68.06% sig-

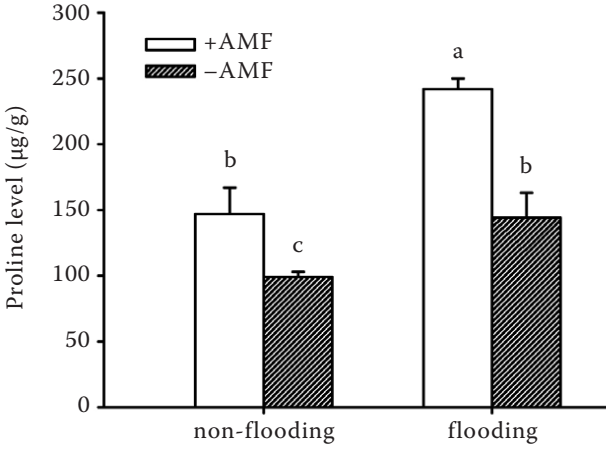

Figure 2. Effects of arbuscular mycorrhizal fungus (AMF) (*Glomus mosseae*) on root proline levels of peach seedlings grown in non-flooding and flooding. Data (means ± standard deviation, n = 3) followed by different letters above the bars among treatments indicate significant differences (P < 0.05)

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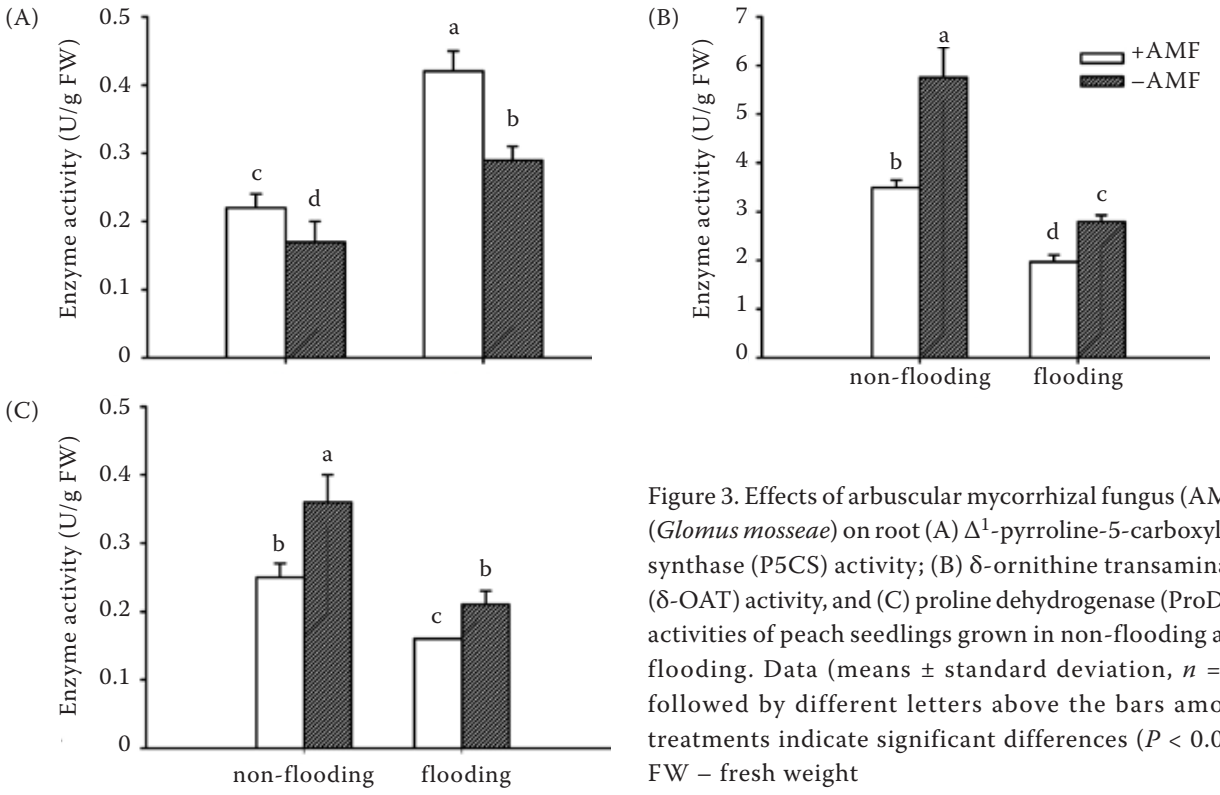

Figure 3. Effects of arbuscular mycorrhizal fungus (AMF) (*Glomus mosseae*) on root (A) Δ^1-pyrroline-5-carboxylate synthase (P5CS) activity; (B) δ-ornithine transaminase (δ-OAT) activity, and (C) proline dehydrogenase (ProDH) activities of peach seedlings grown in non-flooding and flooding. Data (means ± standard deviation, n = 3) followed by different letters above the bars among treatments indicate significant differences (P < 0.05); FW – fresh weight

nificantly higher proline levels than non-mycorrhizal plants under non-flooded and flooded conditions, respectively (Figure 2). This further implies that mycorrhizal plants could further increase root proline accumulation under flooding conditions. Free proline is conducive to cell water retention, and the accumulated proline can reduce the osmotic potential of the protoplasm and prevent the loss of cell water, thereby protecting the protoplasm and retaining water (Garg and Baher 2013). At the same time, proline improves the stability of protoplasmic colloids and keeps physiological metabolism in a stable state (Chun et al. 2018). Therefore, a higher root proline level in mycorrhizal peach trees is essential to improve the flooding tolerance of plants.

Changes in root proline metabolising enzyme activities. The activity of root P5CS was significantly enhanced, and the activity of root δ-OAT was significantly decreased under the flooding (Figure 3A, B). Inoculation of AMF significantly affected the activity of proline metabolism-related enzymes in roots, in which AMF significantly increased the activity of P5CS by 29.41% and 44.83%, but significantly decreased the activity of δ-OAT by 39.30% and 29.39%, respectively, under the conditions of non-flooding and flooding. It suggested that the glutamate synthesis pathway of proline was significantly increased by AMF under both non-flooded and flooded conditions, but the ornithine synthesis pathway of proline was decreased. We concluded that the process of glutamate synthesis pathway, rather than ornithine synthesis pathway, could be significantly accelerated after AMF inoculation in flooded peach, thus promoting the rapid accumulation

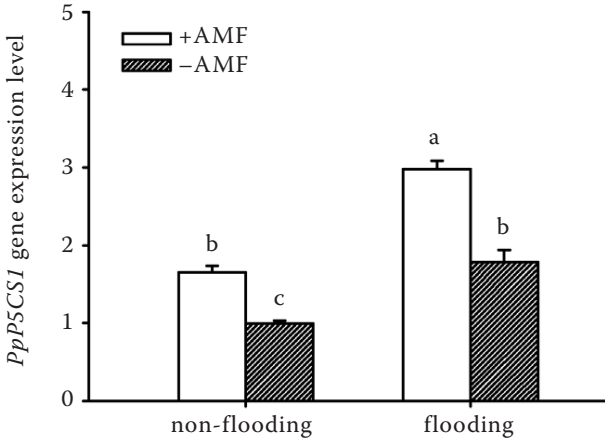

Figure 4. Effects of arbuscular mycorrhizal fungus (AMF) (*Glomus mosseae*) on relative expression levels of root Δ^1-pyrroline-5-carboxylate synthase 1 (*PpP5CS1*) gene of peach seedlings grown in non-flooding and flooding. Data (means ± standard deviation, n = 3) followed by different letters above the bars among treatments indicate significant differences (P < 0.05)

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of proline. After all, proline accumulation in plants is mainly dependent on the glutamate synthesis pathway, not the ornithine synthesis pathway (Wang et al. 2011). Under salt stress, Garg and Baher (2013) also reported that mycorrhizal symbiosis with chickpea roots elevated P5CS activity.

In addition, proline accumulation depended on ProDH catalytic degradation reaction. The results of this study also showed that the ProDH activity of peach roots was significantly decreased under the stress of flooding, and the ProDH activity was significantly decreased by 30.56% and 23.81% after inoculation with AMF (Figure 3C), which was contrary to the change of proline content. Similar results were reported by Garg and Baher (2013) in chickpea under salt stress and *G. mosseae* inoculation. These results indicated that proline accumulation was related to ProDH activity inhibition to a large extent under mycorrhization and flooding conditions.

In a word, the accumulation of proline in flooded peach roots was mainly completed through glutamate synthesis. Inoculation of AMF promoted more accumulation of proline in peach roots exposed to flooding, which was the result of activating glutamate synthesis pathway of proline and inhibiting proline decomposition.

Changes in root *PpP5CS1* gene expression levels. Δ^1-pyrroline-5-carboxylate synthetase (P5CS) is a key enzyme in the glutamate synthesis pathway of proline (Chandrakar and Keshavkant 2018). Earlier studies have confirmed that host *P5CS* gene expression could respond to mycorrhizal fungi under abiotic stress conditions (Porcel et al. 2004, Abo-Doma et al. 2011). In *Glycine max* (L.) Merrill. and *Lactuca sativa* L., *G. mosseae* and *G. intraradices* did not induce the expression of *gmp5cs* and *lsp5cs* gene, and these *P5CS* gene expressions were lower in mycorrhizal than in non-mycorrhizal plants under drought stress (Porcel et al. 2004). In barley (*Hordeum vulgare* L.), AMF inoculation increased the gene expression of *HvP5CS* (Abo-Doma et al. 2011). Our study revealed that flooding stress induced the expression of the *PpP5CS1* gene, irrespective of AMF- and non-AMF-inoculated peach (Figure 4), indicating that the accumulation of proline in flooded peach was related to the up-regulation of the *PpP5CS1* gene. On the other hand, inoculation with *G. mosseae* dramatically up-regulated the *PpP5CS1* gene expression level in peach by 1.65 times and 1.67 times under non-flooding and flooding conditions, respectively (Figure 4). Such induced expression of *PpP5CS1* in mycorrhizal peach under mycorrhization can accumulate more proline in mycorrhizal plants in response to abiotic stress, which is an important mechanism for AMF to enhance host resistance.

In short, inoculation of AMF improved root morphology and promoted proline accumulation in flooded peach, thus enhancing the resistance of mycorrhizal plants to flooding stress. The results provide an important potential for flooding resistance of crops using AMF in the future.

REFERENCES

Abbaspour H., Saeidi-Sar S., Afshari H., Abdel-Wahhab M.A. (2012): Tolerance of mycorrhiza infected pistachio (*Pistacia vera* L.) seedling to drought stress under glasshouse conditions. Journal of Plant Physiology, 169: 704–709.

Abo-Doma A., Edrees S., Abdel-Aziz S. (2011): The effect of mycorrhiza growth and expression of some genes in barley. Egyptian Journal of Genetics and Cytology, 40: 301–313.

Bates L.S., Waldren R.P., Teare I.D. (1973): Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205–207.

Begum N., Qin C., Ahanger M.A., Raza S., Khan M.I., Ashraf M., Ahmed N., Zhang L.X. (2019): Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Frontiers in Plant Science, 10: 1068.

Chandrakar V., Keshavkant S. (2018): Nitric oxide and dimethylthiourea up-regulates pyrroline-5-carboxylate synthetase expression to improve arsenic tolerance in *Glycine max* L. Environmental Progress and Sustainable Energy, 38: 402–409.

Chi G.-G., Srivastava A.K., Wu Q.S. (2018): Exogenous easily extractable glomalin-related soil protein improves drought tolerance of trifoliate orange. Archives of Agronomy and Soil Science, 64: 1341–1350.

Chun S.C., Paramasivan M., Chandrasekaran M. (2018): Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. Frontiers in Microbiology, 9: 2525.

Gao H.F., Peng F.T., Xiao Y.S., Zhang Y.F., Wang G.D., Sun X.W., He Y. (2019): Physiological and biological mechanisms of molybdenum on alleviating chilling stress of peach leaves. Journal of Plant Nutrition and Fertilizers, 25: 1211–1221.

Garg N., Baher N. (2013): Role of arbuscular mycorrhizal symbiosis in proline biosynthesis and metabolism of *Cicer arietinum* L. (chickpea) genotypes under salt stress. Journal of Plant Growth Regulation, 32: 767–778.

Guo C., Huang Y.M., Wu Q.S., Li S. (2015): Effects of AM fungi on proline content and its metabolic enzymes' activity of peach root system under waterlogging stress. Guizhou Agricultural Sciences, 43: 51–53.

He J.D., Chi G.G., Zou Y.N., Shu B., Wu Q.S., Srivastava A.K., Kuča K. (2020): Contribution of glomalin-related soil proteins to soil organic carbon in trifoliate orange. Applied Soil Ecology, 154: 103592.

https://doi.org/10.17221/520/2020-PSE

He J.D., Dong T., Wu H.H., Zou Y.N., Wu Q.S., Kuča K. (2019): Mycorrhizas induce diverse responses of root *TIP* aquaporin gene expression to drought stress in trifoliate orange. Scientia Horticulturae, 243: 64–69.

Kenneth J.L., Schmittgen T.D. (2001): Analysis of relative gene expression data using real-time quantitative PCR and $2^{-\Delta\Delta CT}$ method. Methods, 25: 402–408.

Li S.H. (1993): The response of sensitive periods of fruit tree growth, yield and quality to water stress and water seving irrigation. Plant Physiology Communications, 29: 10–16.

Lin H.H., Lin K.H., Huang M.Y., Su Y.R. (2020): Use of non-destructive measurements to identify cucurbit species (*Cucurbita maxima* and *Cucurbita moschata*) tolerant to waterlogged conditions. Plants, 9: 1226.

Lü L.H., Zou Y.N., Wu Q.S. (2019): Mycorrhizas mitigate soil replant disease of peach through regulating root exudates, soil microbial population, and soil aggregate stability. Communications in Soil Science and Plant Analysis, 50: 909–921.

Ma L.M., Wang P.T., Wang S.G. (2014): Effect of flooding time length on mycorrhizal colonization of three AM fungi in two wetland plants. Environmental Science, 35: 263–270.

Meng L.L., He J.D., Zou Y.N., Wu Q.S., Kuča K. (2020): Mycorrhiza-released glomalin-related soil protein fractions contribute to soil total nitrogen in trifoliate orange. Plant, Soil and Environment, 66: 183–189.

Neto D., Carvalho L.M., Cruz C., Martins-Loução M.A. (2006): How do mycorrhizas affect C and N relationships in flooded *Aster tripolium* plants? Plant and Soil, 279: 51–63.

Osundina M.A. (1998): Nodulation and growth of mycorrhizal *Casuarina equisetifolia* J.R. and G. First in response to flooding. Biology and Fertility of Soils, 26: 95–99.

Phillips J.M., Hayman D.S. (1970): Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55: 158–161.

Porcel R., Azcón R., Ruiz-Lozano J.M. (2004): Evaluation of the role of genes encoding for Δ^1-pyrroline-5-carboxlyate synthetase (P5CS) during drought stress in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants. Physiological and Molecular Plant Pathology, 65: 211–221.

Quan X.Q., Zhang Y.J., Shan L., Bi Y.P. (2007): Advances in proline metabolism researches of higher plants. Biotechnology Bulletin, 1: 14–18.

Wang K., Liu Y.X., Dong K.H., Dong J., Kang J., Yang Q.C., Zhou H., Sun Y. (2011): The effect of NaCl on proline metabolism in *Saussurea amara* seedlings. African Journal of Biotechnology, 10: 2886–2893.

Wang M., Liu D.W., Zeng H.Y., Xiong L., Wu J.B., Qiu L., Zhang W.C., Li L. (2013): Review on waterlogging damage and tolerance mechanism of crop. Crop Research, 27: 284–287.

Wirsel S.G.R. (2004): Homogenous stands of a wetland grass harbour diverse consortia of arbuscular mycorrhizal fungi. FEMS Microbiology Ecology, 48: 129–138.

Wu Q.S., Li G.H., Zou Y.N. (2011): Roles of arbuscular mycorrhizal fungi on growth and nutrient acquisition of peach (*Prunus persica* L. Batsch) seedlings. Journal of Animal and Plant Sciences, 21: 746–750.

Wu Q.S., Srivastava A.K., Zou Y.N. (2013a): AMF-induced tolerance to drought stress in citrus: a review. Scientia Horticulturae, 164: 77–87.

Wu Q.S., Zou Y.N., Huang Y.M. (2013b): The arbuscular mycorrhizal fungus *Diversispora spurca* ameliorates effects of waterlogging on growth, root system architecture and antioxidant enzyme activities of citrus seedlings. Fungal Ecology, 6: 37–43.

Wu H.H., Zou Y.N., Rahman M.M., Ni Q.D., Wu Q.S. (2017): Mycorrhizas alter sucrose and proline metabolism in trifoliate orange exposed to drought stress. Scientific Reports, 7: 42389.

Wu S., Sui X., Zhang T., Chen Y.T., Zhu D.G., Cui F.X., Yang L.B. (2019a): Research on the progress of AMF in wetland. Territory and Natural Resources Study, 6: 80–84.

Wu Q.S., He J.D., Srivastava A.K., Zou Y.N., Kuča K. (2019b): Mycorrhizas enhance drought tolerance of citrus by altering root fatty acid compositions and their saturation levels. Tree Physiology, 39: 1149–1158.

Xiao Y.S. (2015): Effects of aeration cultivation on root architecture and the growth of peach trees. [Ph.D. Thesis] Tai-an, Shandong Agricultural University, 1–103.

Xie M.M., Zou Y.N., Wu Q.S., Zhang Z.Z., Kuča K. (2020): Single or dual inoculation of arbuscular mycorrhizal fungi and rhizobia regulates plant growth and nitrogen acquisition in white clover. Plant, Soil and Environment, 66: 287–294.

Zhang F., Zou Y.N., Wu Q.S., Kuča K. (2020): Arbuscular mycorrhizas modulate root polyamine metabolism to enhance drought tolerance of trifoliate orange. Environmental and Experimental Botany, 171: 103926.

Zhao F.G., Sun C., Liu Y.L., Zhang W.H., Liu Z.P. (2002): Effects of ABA and NaCl on metabolism of polyamines and proline in *Suaeda glauca* Bunge. Journal of Plant Physiology and Molecular Biology, 28: 117–120.

Zou Y.N., Srivastava A.K., Wu Q.S. (2016): Glomalin: a potential soil conditioner for perennial fruits. International Journal of Agriculture and Biology, 18: 293–297.

Zou Y.N., Srivastava A.K., Wu Q.S., Huang Y.M. (2014): Increasing tolerance of trifoliate orange (*Poncirus trifoliata*) seedlings to waterlogging after inoculation with arbuscular mycorrhizal fungi. Journal of Animal and Plant Sciences, 24: 1415–1420.

Zou Y.N., Wu Q.S., Huang Y.M., Ni Q.D., He X.H. (2013): Mycorrhizal-mediated lower proline accumulation in *Poncirus trifoliata* under water deficit derives from the integration of inhibition of proline synthesis with increase of proline degradation. PLoS ONE, 8: e80568.

Zou Y.N., Wu Q.S., Kuča K. (2020): Unraveling the role of arbuscular mycorrhizal fungi in mitigating the oxidative burst of plants under drought stress. Plant Biology. doi.org/10.1111/plb.13161

Received: October 9, 2020
Accepted: November 9, 2020
Published online: November 16, 2020