Emirates Journal of Food and Agriculture. 2015. 27(10): 763-769
doi: 10.9755/ejfa.2015.05.240
http://www.ejfa.me/

REGULAR ARTICLE

Alleviation of magnesium deficiency by mycorrhiza in trifoliate orange: Changes in physiological activity

Fei Zhang[1,2], Peng Du[1], Chang-Xu Song[1], Qiang-Sheng Wu[1,2]*

[1]College of Horticulture and Gardening, Yangtze University, Jingzhou, Hubei 434025, China; [2]Institute of Root Biology, Yangtze University, Jingzhou, Hubei 434025, China

ABSTRACT

Deficiency of magnesium (Mg) is quite common in citrus trees and often results in leaf chlorosis. In this study, five-leaf-old trifoliate orange (*Poncirus trifoliata*) seedlings were grown in sands and inoculated with an arbuscular mycorrhizal fungus (AMF), *Funneliformis mosseae.* After 8 days of transplanting, the seedlings were subjected to Mg-deficient (0.2 mM Mg^{2+}) and Mg-sufficient (2 mM Mg^{2+}) treatments for 92 days. Growth performance, chlorophyll concentration, soluble protein concentration, and antioxidant enzyme activities were determined. The results showed that root AMF colonization was significantly higher under Mg-deficiency than under Mg-sufficiency. AMF inoculation significantly increased leaf, stem, root, and total (leaf + stem + root) biomass production and third-order lateral root number than non-AMF treatment under Mg-deficient and Mg-sufficient conditions. Compared with Mg-sufficiency, Mg-deficiency considerably reduced chlorophyll *a* and chlorophyll *a* + *b* concentration, whereas AMF inoculation significantly increased chlorophyll *a* and chlorophyll *a* + *b* concentration, representing the alleviation of leaf chlorosis by mycorrhization. The AMF seedlings presented significantly higher concentration of soluble protein concentration in leaf and root and activity of superoxide dismutase and catalase in root than the non-AMF seedlings exposed to Mg-deficient and Mg-sufficient, respectively. It concludes that AMF inoculation had positive effects on growth performance and physiological activities of trifoliate orange under Mg deficient condition, thus, possibly enhancing tolerance to Mg deficiency.

Keywords: Antioxidation; Arbuscular mycorrhizal fungi; Citrus; Lateral root; Nutrient deficiency

INTRODUCTION

Magnesium (Mg) is needed for many physiological processes in plants, particularly chlorophyll production, because it is an essential constituent of chlorophyll. Without a sufficient Mg level in soil and plant tissue, plants usually exhibit leaf chlorosis, symptom of Mg deficiency. In addition, Mg is a key activator for many critical enzymes in physiological activities. Deficiency of Mg is quite common in citrus trees in China and USA (Razeto and Salas, 1986), primarily due to low soil Mg concentration. Hence, enhancing tolerance of citrus trees to Mg deficiency in soil is an increasing gap in citrus production.

Arbuscular mycorrhizal fungi (AMF), one of the important soil beneficial fungi, can form symbiotic associations with the roots of ~80% of plants in terrestrial ecosystems, including citrus plants (Fidelibus et al., 2001; Wu et al., 2013a; Ortas and Ustuner, 2014). AMF develop wide extraradical mycorrhizal network into rhizosphere of the host plant, which enlarges the root contacted areas of water and nutrient absorption in soil, but also stabilizes soil structure (Bedini et al., 2009). Therefore, AMF play a prominent role in nutrient uptake, including Zn, P, and Cu, from the soil to the fungal partner (Meier et al., 2012; Lehmann et al., 2014). Spraying high levels of Mg on leaves strongly inhibited root AM colonization and sporulation in sweet potato and onion grown in aeroponic and sand culture (Jarstfer et al., 1998), suggesting a negative correlation between Mg level and root AM colonization. A pot study revealed that the plants (*Knautia arvensis* and *Dipsacaceae*) inoculated with different *Glomus* species strongly promoted P uptake, but also increased Mg uptake in serpentine soils (Doubkova et al., 2012). In *Citrus tangerina* plants, inoculation with *Diversispora versiformis* significantly increased leaf Mg level under well-watered and drought stress conditions (Wu and Xia, 2006). In addition, AMF inoculation considerably enhanced antioxidant enzyme activities in citrus plants exposed to salt stress, temperature stress, and waterlogging stress (Wu, 2011; Wu et al., 2013b;

***Corresponding author:**
Qiang-Sheng Wu, College of Horticulture and Gardening, Yangtze University, Jingzhou, Hubei 434025, China, Tel/Fax: 86 716 8066262, E-mail: wuqiangsh@163.com

Received: 11 May 2015; **Revised:** 28 June 2015; **Accepted:** 16 July 2015; **Published Online:** 16 July 2015

Zou, 2011). These studies indicated the critical roles of AM symbiosis in tolerance of the host plants to abiotic stresses. Nevertheless, the information about mycorrhizal effects on growth and antioxidant enzyme activities in citrus plants is still poorly understood.

The objective of the present study was to evaluate the effects of AMF inoculation on plant biomass, chlorophyll concentration, soluble protein concentration, and antioxidant enzymatic activities in trifoliate orange [*Poncirus trifoliata* (L.) Raf.] under Mg deficient and Mg sufficient conditions.

MATERIALS AND METHODS

Plant culture

Seeds of trifoliate orange, collected from a citrus orchard of Yangtze University campus, were germinated in the autoclaved (0.11 Mpa, 121°C, 2 h) sands in a growth chamber (26/20°C day/night temperature, 740 μmol/m^2/s photon flux density, and 80% relative humidity). After 21 days, four five-leaf-old seedlings with uniform size were transplanted into a plastic pot with the size of 17 cm upper diameter, 13 cm bottom diameter, and 16 cm height, where 2.5 kg of autoclaved sand (<4 mm size) was supplied.

At the time of transplanting, the sand substrate was mixed with and without *Funneliformis mosseae* (Nicol. & Gerd.) Schüßler & Walker. Approx. 2000 spores per pot was inoculated, but the non-AMF inoculated pot received the autoclaved inocula plus 3 mL filtrate (25 μm) of inocula to keep similar microbial communities except *F. mosseae*. The strain of *F. mosseae* was isolated from the rhizosphere of *Incarvillea younghusbandii* in Dangxiong, Tibet, China. The AM strain was propagated with pot culture in *Trifolium repens* as a host plant for 16 weeks.

All the treated seedlings were placed in a glass house from March 30 to August 2, 2014, where photo flux density is 982 μmol/m^2/s, day/night temperature 27/20°C, and relative air humidity 80%.

Experimental design

The experiment was arranged in a 2^2 completely randomized blocked design, which consisted of inoculation with or without *F. mosseae* and Mg treatments (0.2 or 2 mM Mg level in growth substrate). Each treatment replicated four times, resulting in a total of 16 pots. Meanwhile, 2 mM $MgSO_4$, a Mg-sufficient (Mg-S) level in the substrate, is the stardand Hoagland nutrient solution and is relatively fit for growth in trifoliate orange. The 0.2 mM Mg level (corresponding to 1/10 strength stardand Hoagland nutrient solution) was designed as Mg-deficient (Mg-D) level in the substrate. After 8 days of seedlings transplanting, all the seedlings were watered daily by 100 mL Hoagland solution per pot, in company with 0.2 and 2 mM $MgSO_4$ as the treated level for corresponding pot until the harvest. The Mg strength treatments were kept for 92 days after transplanting.

Measurements of variables

At harvest, the seedlings were divided into leaf, stem, and root, whose fresh weight was determined and recorded, subsequently. The numbers of different order lateral roots were counted.

A number of 1-cm-length root segments (30 root segments per treatment) were cleared by 10% (w/v) KOH solution for 90 min at 95°C and stained with 0.05% (w/v) trypan blue in lactoglycerol (Phillips and Hayman, 1970). The root AM colonization was expressed as the percentage of AMF colonized root length against observed total root length.

Leaf chlorophyll concentration was determined according to the method of Lichtenthaler and Wellburn (1983), based on the extraction of 80% acetone.

Fresh samples (0.20 g) of leaf and root were homogenized in 5 mL 0.1 M phosphate buffer (pH 7.8) and centrifuged at 4,200×*g* for 10 min at 4°C. The supernatants were used for the biochemical assays. Leaf and root soluble protein concentrations were determined by the protocol of Bradford (1976) using bovine serum albumin as a standard. Superoxide dismutase (SOD) activity in leaves and roots was monitored according to Giannopolitis and Ries (1977). One unit of SOD was expressed as the amount of enzyme inhibiting 50% nitro blue tetrazolium by light. Catalase (CAT) activity in leaves and roots was determined by the method previously described by Wu et al. (2010). Simply, 2.5 mL 0.1 M H_2O_2 was added into 2.5 mL supernatant at 30°C for 10 min. Subsequently, the chemical reaction was stopped by adding 2.5 mL 10% H_2SO_4. The residual H_2O_2 was titrated with 0.1 M $KMnO_4$. The unit of CAT activity was defined as mg H_2O_2 decomposion per g FW per min.

Statistical analysis

Data (means ± SD, n=4) were statistically analyzed with variance (ANOVA) in SAS (SAS Institute Inc., Cary, NC, USA), and significant differences among treatments were compared by the Duncan's multiple range tests at $P < 0.05$.

RESULTS AND DISCUSSION

Root AM colonization

In the present study, root mycorrhizal colonization was not found in the non-AMF inoculated trifoliate orange

seedlings, but varied from 60.03 to 88.25% in the AMF seedlings (Table 1 and Fig. 1a–1b). Meanwhile, the Mg-D treatment represented 32% significantly higher root AM colonization than the Mg-S treatment. The result was in agreement with the findings of Ardestani et al. (2011) and Liu et al. (2000), who observed the decrease in root colonization with the increasing of soil nutrient levels. Jarstfer et al. (1998) found the decline in root colonization and sporulation in sweet potato and onion colonized by *Glomus* sp. under high Mg level conditions. Therefore, Mg may have a specific biological effect on root AM colonization (Gryndler et al., 1992).

Plant growth performance

Our results showed that the Mg-D treatment significantly reduced leaf, stem, root, and total fresh weights of the trifoliate orange seedlings than the Mg-S treatment, irrespective of AMF inoculated or not (Table 1). However, compared with the non-AMF inoculated seedlings, the AMF treated seedlings showed 85, 34, 20, and 38% significantly higher leaf, stem, root and total fresh weights under the Mg-D treatment conditions and 69, 27, 21, and 32% higher under the Mg-S treatment conditions. This result is consistent with previous studies in trifoliate orange seedlings exposed to waterlogging stress (Wu et al., 2013b), drought stress (Huang et al., 2014), and salt stress (Wu et al., 2010). These results imply that AMF possessed the potential capacity to promote plant growth under various soil stresses conditions.

The present study indicated that Mg deficient treatment did not significantly alter the number of lateral roots in 1st, 2nd, and 3rd order, except that a significantly higher 2nd order lateral root number was found in non-AMF seedlings under Mg-D than under Mg-S conditions (Table 1). In addition, the AMF colonization significantly increased number of 3rd order lateral root by 100% under Mg-D conditions and number of 2nd and 3rd order lateral root by 54 and 150% under Mg-S conditions, respectively. The number of 1st order lateral root was not altered by mycorrhization with *F. mosseae*. Zou et al. (2014) reported that the trifoliate orange plants colonized by *Diversispora spurca* represented greater lateral root number than non-AMF colonized plants under waterlogging and non-waterlogging conditions. It is known that number of lateral root is a major factor to affect tolerance to abiotic stresses (Remans et al., 2012). Therefore, considerably greater lateral root number in AM plants would promote nutrient uptake, thus, enhancing tolerance of mycorrhizal seedlings to Mg stress.

Chlorophyll concentrations

Our result indicated that the Mg-D treatment generally decreased leaf chlorophyll *a* and chlorophyll *a*+*b* concentration in AM and non-AM seedlings, significantly decreased chlorophyll *b* in AM seedlings, but did not affect carotenoid concentration in AM and non-AM seedlings, as compared with the Mg-S treatment (Fig. 2). It seems that Mg deficiency led to an obvious decline in chlorophyll concentration and thus resulted in leaf chlorosis in citrus plants (Ling et al., 2014), because Mg is the central atom of chlorophyll molecule. When the trifoliate orange seedlings were inoculated with *F. mosseae*, these AM seedlings represented 27, 57, and 22% significantly higher chlorophyll *a*, carotenoid, and chlorophyll *a*+*b* concentrations under the Mg-D conditions, and 34, 60, and 42% significantly higher chlorophyll *a*, chlorophyll *b*, and chlorophyll *a*+*b*

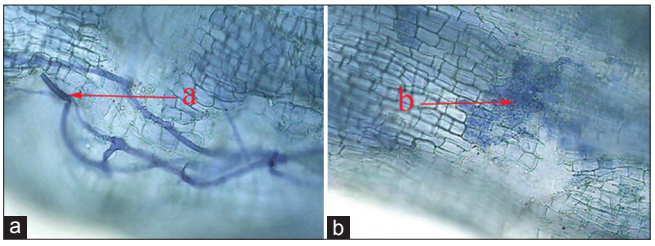

Fig 1. Root AM colonization of trifoliate orange [*Poncirus trifoliata* (L.) Raf] seedlings by *Funneliformis mosseae* under Mg stress conditions. Herein, 'a' indicated mycorrhizal colonization in root cortical cells, and 'b' showed plentiful arbuscles in root cortical cells.

Table 1: Root mycorrhizal colonization, leaf, stem, root, and total (leaf+stem+root) biomass and number of lateral roots in *Funneliformis mosseae*-colonized trifoliate orange [*Poncirus trifoliata* (L.) Raf.] seedlings grown under 0.2 mM Mg (Mg-deficiency, Mg-D) and 0.2 mM Mg (Mg-sufficiency, Mg-S) conditions

Mg treatment	AMF status	Root AM colonization (%)	Biomass (g FW/plant)				Number of lateral roots (num./plant)		
			Leaf	Stem	Root	Total	1st	2nd	3rd
Mg-D	–AMF	0±0c	0.67±0.10d	1.54±0.14c	1.26±0.15d	3.48±0.35c	43±2a	92±6a	9±4b
	+AMF	88.25±1.07a	1.24±0.06b	2.06±0.28ab	1.51±0.15c	4.81±0.36b	41±4a	93±6a	18±4a
Mg-S	–AMF	0±0c	0.87±0.08c	1.82±0.25bc	2.16±0.12b	4.85±0.20b	42±3a	59±4b	6±2b
	+AMF	60.03±1.03b	1.47±0.10a	2.31±0.11a	2.61±0.13a	6.39±0.25a	40±6a	91±6a	15±2a
Significance									
Mg treatment		**	**	*	**	**	NS	**	NS
AMF		**	**	**	**	**	NS	**	**
Interaction		**	NS	NS	NS	NS	NS	**	NS

Note: Data (means±SD, *n*=4) followed by different letters between the treatments indicate significant differences at 5% level. NS: Not significant. *P<0.05. **P<0.01

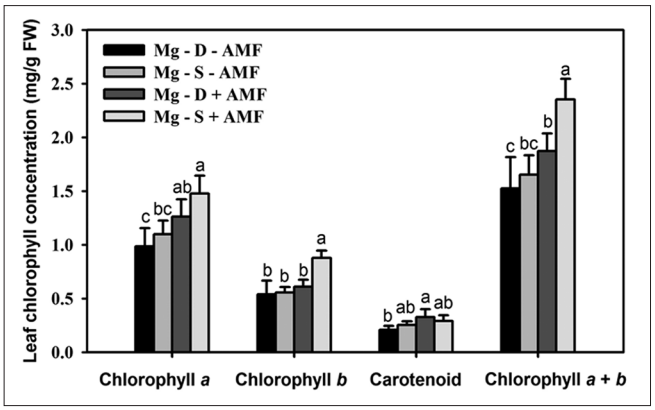

Fig 2. Effect of *Funneliformis mosseae* on chlorophyll *a*, chlorophyll *b*, chlorophyll *a*+*b*, and carotenoid concentration in trifoliate orange [*Poncirus trifoliata* (L.) Raf.] seedlings grown under 0.2 mM Mg (Mg-deficiency, Mg-D) and 0.2 mM Mg (Mg-sufficiency, Mg-S) conditions. Data (means ± SD, $n = 4$) are significantly different ($P<0.05$) if followed by different letters above the bars.

concentrations under the Mg-S conditions. Studies had shown the increase of Mg in leaves and roots of the host plants including tomato and citrus after AMF inoculation (El-Shanshoury et al., 1989; Xiao et al., 2014). We concluded that due to absorption of mycorrhizal hyphae to Mg element from substrate to fungal partner, AM seedlings may have greater Mg level in leaves, thereby, benefiting the synthesis of chlorophyll under different Mg levels.

In addition, AM seedlings had 57% significantly higher carotenoid concentration than non-AM seedlings under the Mg-D conditions, but not under the Mg-S conditions (Fig. 2). It seems that the AM effect on carotenoid is more important in Mg-D than in Mg-S. In fact, carotenoids can protect photosynthetic apparatus against oxidative damage, as well quench excited triple state of chlorophyll in plants (Asrar and Elhindi, 2011.). Xiao et al. (2014) confirmed greater photosynthesis of Newhall and Ponkan seedlings inoculated with *Glomus versiforme* under Mg-D conditions. Therefore, under Mg-D conditions, AM plants possessed higher carotenoids to protect against oxidative damage.

Soluble protein

In the present study, the Mg-deficient treatment significantly induced the decrease of soluble protein concentration in leaves and roots, compared with the Mg-S treatment. On the other hand, inoculation with AMF significantly increased leaf and root soluble protein concentration by 15 and 11% under the Mg-D conditions and by 28 and 66% under the Mg-S conditions, respectively (Fig. 3a–3b). Possibly, AMF decreased protein degradation, or induce new protein production to cope with the abiotic stress (Tang et al., 2009).

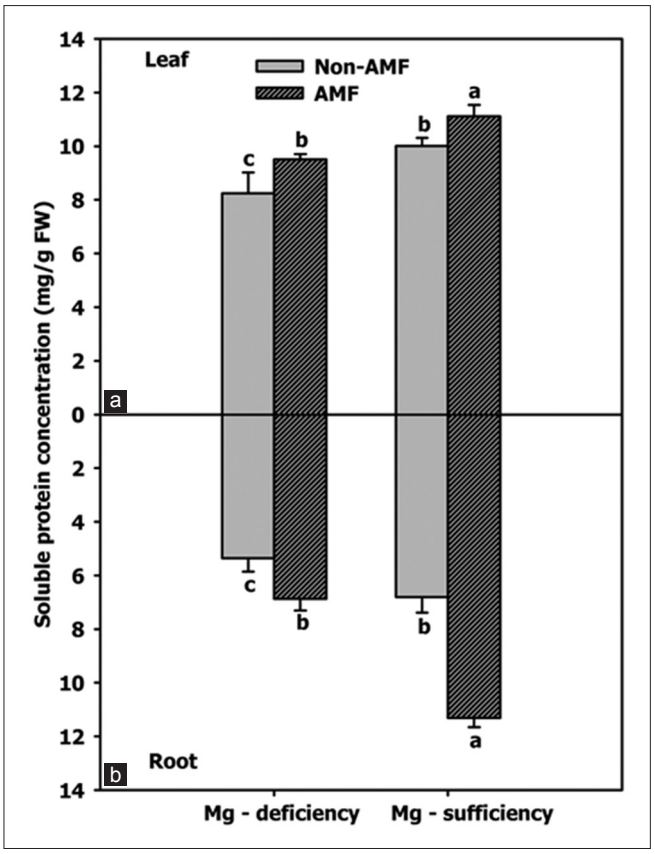

Fig 3. Effect of *Funneliformis mosseae* on leaf (a) and root (b) soluble protein concentration in trifoliate orange [*Poncirus trifoliata* (L.) Raf.] seedlings grown under 0.2 mM Mg (Mg-deficiency, Mg-D) and 0.2 mM Mg (Mg-sufficiency, Mg-S) conditions. Data (means ± SD, $n = 4$) are significantly different ($P<0.05$) if followed by different letters above the bars.

Antioxidant enzyme activities

SOD can catalyze the dismutation of $O_2^{\bullet-}$ to H_2O_2 (Mohammadkhani and Heidari, 2008). In the present study, low Mg treatment did not alter leaf SOD activity in non-AM seedlings, but significantly decreased leaf SOD activity in AM seedlings (Fig. 4a). In roots, Mg deficiency significantly decreased SOD activity, regardless of AM or non-AM seedlings (Fig. 4b). It implies that low Mg stress could decrease the antioxidant enzyme protective system to deal with the oxidative burst, resulting in a greater oxidative damage. AM seedlings showed 61 and 16% significantly higher leaf and root SOD activity under the Mg-S conditions and 27% significantly higher root SOD activity under the Mg-D treatment (Fig. 4a–4b). It seems that roots preferred to cope with the oxidative damage, because roots firstly contact the substrate Mg stress. This result is consistent with the results reported for *Olea europaea, Suaeda salsa*, soybean, and tomato (Alguacil et al., 2003; Li et al., 2012; He et al., 2007). SOD as protective enzyme can contribute in synergy to removing excessive reactive oxygen species in plants, thus decreasing stress damage

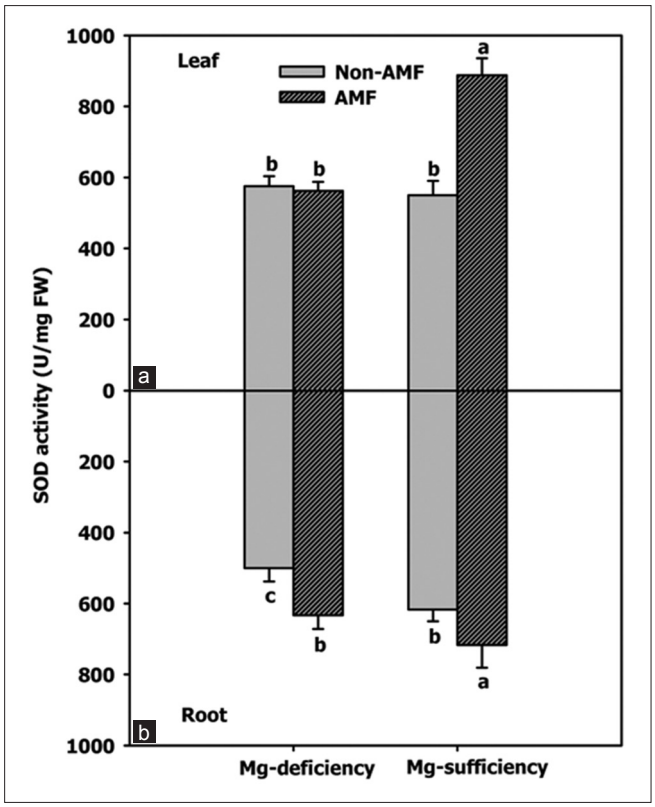

Fig 4. Effect of *Funneliformis mosseae* on leaf (a) and root (b) superoxide dismutase (SOD) activity in trifoliate orange [*Poncirus trifoliata* (L.) Raf.] seedlings grown under 0.2 mM Mg (Mg-deficiency, Mg-D) and 0.2 mM Mg (Mg-sufficiency, Mg-S) conditions. Data (means ± SD, $n = 4$) are significantly different ($P<0.05$) if followed by different letters above the bars.

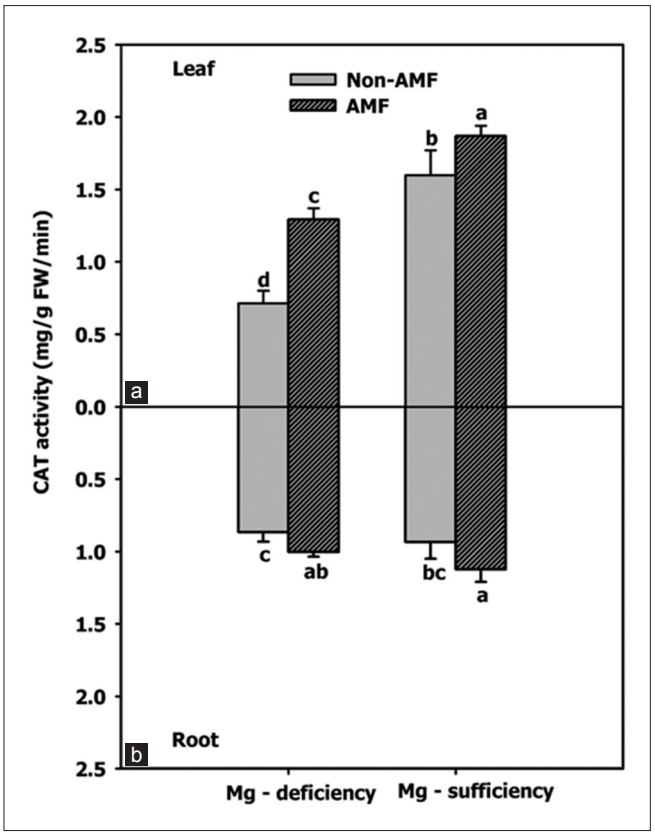

Fig 5. Effect of *Funneliformis mosseae* on leaf (a) and root (b) catalase (CAT) activity in trifoliate orange [*Poncirus trifoliata* (L.) Raf.] seedlings grown under 0.2 mM Mg (Mg-deficiency, Mg-D) and 0.2 mM Mg (Mg-sufficiency, Mg-S) conditions. Data (means ± SD, $n = 4$) are significantly different ($P<0.05$) if followed by different letters above the bars.

to a certain extent (Zushi et al., 2009; Abed et al., 2001). The present study indicated that AM roots might possess higher SOD activity to eliminate reactive oxygen species, thus, enhancing tolerance to Mg deficiency.

CAT is one of the most important antioxidant enzymes that eliminates H_2O_2 from plant cells (Abbaspour et al., 2012). Tian et al. (2013) reported that inoculation with AMF significantly increased leaf CAT activity in *Plukenetia volubilis* seedlings, thus, alleviating oxidative damage by drought stress. Our results showed that although the Mg deficiency significantly restricted leaf and root CAT activity in AM and non-AM seedlings, CAT activity in mycorrhizal seedlings was 82 and 15% higher in leaf and root, respectively, under the Mg-D conditions, and 17 and 19% higher in leaf and root under the Mg-S conditions (Fig. 5a–5b). This suggests that greater CAT activity in tissues of AM plants might possess a greater capacity to eliminate H_2O_2, thereby, alleviating the oxidative damage. As a result, inoculation with *F. mosseae* enhanced the tolerance of the host plant to low Mg treatment, in terms of greater CAT activity in AM seedlings.

CONCLUSIONS

In conclusion, the low Mg stress considerably inhibited plant growth performance and activity of antioxidant enzymes (SOD and CAT) in trifoliate orange seedlings, as compared with the sufficient Mg treatment. After inoculation with an AMF, *F. mosseae*, the AM seedlings displayed greater growth performance, and significantly higher chlorophyll *a* and chlorophyll *a+b* concentration, soluble protein concentration, and SOD and CAT activity under the Mg-D and the Mg-S conditions, suggesting that AM plants possess greater physiological activities under Mg deficient conditions.

ACKNOWLEDGEMENTS

This study was supported by the Open Fund of Institute of Root Biology, Yangtze University (R201401).

Author contributions

C-X. S and P. D designed the study and took the data. F. Z did the data analysis and wrote this paper. Q.S. W supervised the research project and also corrected the article.

REFERENCES

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

Abed, S. and M. N. Peter. 2001. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J. Exp. Bot., 52: 2207-2211.

Alguacil, M. M., J. A. Hernandez, F. Caravaca, B. Portillo and A. Roldan. 2003. Antioxidant enzyme activities in shoots from three mycorrhizal shrub species afforested in a degraded semi-arid soil. Physiol. Plant. 118: 562-570.

Ardestani, N. K., H. Zare-Maivan and F. Ghanati. 2011. Effect of different concentrations of potassium and magnesium on mycorrhizal colonization of maize in pot culture. Afr. J. Biotechnol. 10: 16548-16550.

Asrar, A. W. A. and K. M. Elhindi. 2011. Alleviation of drought stress of marigold (*Tagetes erecta*) plants by using arbuscular mycorrhizal fungi. Saudi J. Biol. Sci. 18: 93-98.

Bedini, S., E. Pellegrino, L. Avio, S. Pellegrini, P. Bazzoffi, E. Argese and M. Giovannetti. 2009. Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. Soil Biol. Biochem. 41: 1491-1496.

Doubkova, P., J. Suda and R. Sudova. 2012. The symbiosis with arbuscular mycorrhizal fungi contributes to plant tolerance to serpentine edaphic stress. Soil Biol. Biochem. 44: 56-64.

El-Shanshoury, A. R., M. A. Hassan and B. A. Abdel-Ghaffar. 1989. Synergistic effect of vesicular-arbuscular mycorrhizas and *Azotobacter chroococcum* on the growth and the nutrient contents of tomato plants. Phyton. 29: 203-212.

Fidelibus, M. W., C. A. Martin and J. C. Stutz. 2001. Geographic isolates of *Glomus* increase root growth and whole-plant transpiration of *Citrus* seedlings grown with high phosphorus. Mycorrhiza. 10: 231-236.

Giannopolitis, C. N. and S. K. Ries. 1977. Superoxide dismutase. I. Occurrence in higher plants. Plant Physiol. 59: 309-314.

Gryndler, M., H. Vejsadova and V. Vancura. 1992. The effect of magnesium ions on the vesicular-arbuscular mycorrhizal infection of maize roots. New Phytol. 122: 455-460.

He, X. H. and K. Nara. 2007. Element biofortification: Can mycorrhizas potentially offer a more effective and sustainable pathway to curb human malnutrition? Trends Plant Sci. 12: 331-333.

Huang, Y. M., A. K. Srivastava, Y. N. Zou, Q. D. Ni, Y. Han and Q. S. Wu. 2014. Mycorrhizal-induced calmodulin mediated changes in antioxidant enzymes and growth response of drought-stressed trifoliate orange. Front. Microbiol. 5: 682.

Jarstfer, A. G., P. Farmer-Koppenol and D. M. Sylvia. 1998. Tissue magnesium and calcium affect arbuscular mycorrhiza development and fungal reproduction. Mycorrhiza. 7: 237-242.

Lehmann, A., S. D. Veresoglou, E. F. Leifheit and M. C. Rillig. 2014. Arbucular mycorrhizal influence on zinc nutrition in crop plants - A meta-analysis. Soil Biol. Biochem. 69: 123-131.

Li, T., R. J. Liu, X. H. He and B. S. Wang. 2012. Enhancement of superoxide dismutase and catalase activities and salt tolerance of euhalophyte *Suaeda salsa* L. by mycorrhizal fungus *Glomus mosseae*. Pedosphere. 22: 217-224.

Lichtenthaler, H. K. and A. R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans., 11: 591-592.

Ling, L. L., Y. Huang, L. Z. Peng, Y. T. Wu, C. L. Jiang, L. Cao and C. P. Chun. 2014. Influence of magnesium deficiency and excess on chlorophlly fluorescence characteristics of Newhall navel orange leaves. Acta Ecol. Sin. 34: 1672-1680.

Liu, A., C. Hamel, R. I. Hamilton, B. L. Ma and D. L. Smith. 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza. 9: 331-336.

Meier, S., F. Borie, G. Curaqueo, N. Bolan and P. Cornejo. 2012. Effects of arbuscular mycorrhizal inoculation on metallophyte and agricultural plants growing at increasing copper levels. Appl. Soil Ecol. 61: 280-287.

Mohammadkhani, N. and R. Heidari. 2008. Effects of drought stress on soluble proteins in two maize varieties. Turk. J. Biol. 32: 23-30.

Ortas, I. and O. Ustuner. 2014. Determination of different growth media and various mycorrhizae species on citrus growth and nutrient uptake. Sci. Hortic. 166: 84-90.

Phillips, J. M. and D. S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55: 158-161.

Razeto, B. and A. Salas. 1986. Magnesium, manganese and zinc sprays on orange trees (*Citrus sinensis* (L.) Osbeck). In: Alexander, A (Ed.), Foliar Fertilization. Springer, Netherlands, Pp. 255-270.

Remans, T., S. Thijs, S. Truyens, N. Weyens, K. Schellingen, E. Keunen, H. Gielen, A. Cuypers and J. Vangronsveld. 2012. Understanding the development of roots exposed to contaminants and the potential of plant-associated bacterial for optimization of growth. Ann. Bot. 110: 239-252.

Tang, M., H. Chen, J. C. Huang, Z. Q. Tian. 2009. AM fungi effects on the growth and physiology of *Zea mays* seedlings under diesel stress. Soil Biol. Biochem. 41: 936-940.

Tian, Y. H., Y. B. Lei, Y. L. Zheng and Z. Q. Cai. 2013. Synergistic effect of colonization with arbuscular mycorrhizal fungi improves growth and drought tolerance of *Plukenetia volubilis* seedlings. Acta Physiol. Plant. 35: 687-696.

Wu, Q. S. 2011. Mycorrhizal efficacy of trifoliate orange seedlings on alleviating temperature stress. Plant Soil Environ. 57: 459-464.

Wu, Q. S., A. K. Srivastava and Y. N. Zou. 2013a. AMF-induced tolerance to drought stress in citrus: A review. Sci. Hortic. 164: 77-87.

Wu, Q. S., R. X. Xia and Y. N. Zou. 2006. Reactive oxygen metabolism in mycorrhizal and non -mycorrhizal citrus (*Poncirus trifoliata*) seedlings subjected to water stress. J. Plant Physiol. 163: 1101-1110.

Wu, Q. S., Y. N. Zou and Y. M. Huang. 2013b. The arbuscular mycorrhizal fungus *Diversispora spurca* ameliorates effects of waterlogging on growth, root system, architecture and antioxidant enzymes activities of citrus seedings. Fungal Ecol. 6: 37-43.

Wu, Q. S., Y. N. Zou, W. Liu, X. F. Ye, H. F. Zai and L. J. Zhao. 2010. Alleviation of salt stress in citrus seedlings inoculated with mycorrhiza: Changes in leaf antioxidant defense systems. Plant Soil Environ. 56: 470-475.

Xiao, J. X., C. Y. Hu, Y. Y. Chen, B. Yang and J. Hua. 2014. Effects of low magnesium and an arbuscular mycorrhizal fungus on the growth, magnesium distribution and photosynthesis of two citrus cultivars. Sci. Hortic. 177: 14-20.

Zou, Y. N. and Q. S. Wu. 2011. Efficiencies of five arbuscular mycorrhizal fungi in alleviating salt stress of trifoliate orange. Int. J Agric. Biol. 13: 991-995.

Zou, Y. N., A. K. Srivastava, Q. S. Wu and Y. M. Huang. 2014. Increased tolerance of trifoliate orange (*Poncirus Trifoliata*) seedings to waterlogging after inoculation with arbuscular mycorrhizal fungi. J. Anim. Plant Sci. 24: 1415-1420.

Zushi, K., N. Matsuzoe and M. Kitano. 2009. Developmental and tissue-specific changes in oxidative parameters and antioxidant systems in tomato fruits grown under salt stress. Sci. Hortic. 122: 362-368.