Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2019, 47(4):1161-1167. DOI:10.15835/nbha47411561

Original Article

Mycorrhiza Regulates Signal Substance Levels and Pathogen Defense Gene Expression to Resist Citrus Canker

Miao-Miao XIE[1], Yi-Can ZHANG[1], Li-Ping LIU[1], Ying-Ning ZOU[1]*, Qiang-Sheng WU[1,2]*, Kamil KUČA[2]

[1]*Yangtze University, College of Horticulture and Gardening, Jingzhou, Hubei 434025, China; 972948575@qq.com; zhangyic1992@163.com; llp31302@163.com; zouyingning@163.com (*corresponding author); wuqiangsh@163.com (*corresponding author)*

[2]*University of Hradec Kralove, Faculty of Science, Department of Chemistry, Hradec Kralove 50003, Czech Republic; kamil.kuca@uhk.cz*

Abstract

Citrus canker is a quarantined disease, severely harming citrus plants. Soil beneficial arbuscular mycorrhizal fungi (AMF) can provide a biological control pathway to resist pathogens. This work was to test changes of signal substances including hydrogen peroxide (H_2O_2), nitric oxide (NO), calmodulin (CaM), salicylic acid (SA) and jasmonic acid (JA) and the pathogen defense gene expression in roots of AMF (*Paraglomus occultum*) and non-AMF trifoliate orange (*Poncirus trifoliata*) seedlings after infected by a expressions citrus canker pathogen (*Xanthomonas axonopodis* pv. *Citri*, Xac). AMF inoculation significantly improved plant height, stem diameter and leaf number. Xac infection dramatically decreased root H_2O_2, NO, and SA levels, but increased root CaM and JA concentrations in non-AMF seedlings. There were higher H_2O_2 and CaM levels and lower JA levels in Xac-infected seedlings than in non-Xac-infected seedlings under mycorrhization. Under non-Xac infection, mycorrhizal treatment reduced root H_2O_2, NO, and SA but increased CaM and JA levels. However, under Xac infection, mycorrhizal inoculation distinctly accelerated root H_2O_2, NO, CaM, and SA accumulation, accompanied with up-regulated expression levels of root *PtEPS1* (enhance pseudomonas susceptibility 1) and *PtPR4* (pathogenesis related gene 4), indicating that Xac stimulated mycorrhizal roles in enhancing resistance of citrus canker. Such results imply that citrus plants with pre-inoculated AMF had stronger resistance to Xac infection through increasing signal substrate accumulation and pathogen defense gene expressions.

Keywords: arbuscular mycorrhizal fungi; canker; pathogenesis related gene; salicylic acid

Introduction

Citrus is the most popular fruit in China, where citrus planted area is the biggest (about 22% of total fruit areas) and the yield is account for 15% of total fruit yield in 2017. In open field, citrus plants are often subjected to various diseases and insect pests, whilst citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (Xac) is a quarantined disease in the world, severely decreasing tree growth and fruit production (Das, 2003). Until now, there is no effective way to prevent the disease.

Arbuscular mycorrhizal (AM) fungi (AMF), a kind of soil beneficial microorganisms, establish the symbiotic association with roots to enhance biotic resistance of host plants including root-knot nematode, bacterioses and fungal disease (Liu *et al*., 2007; Nair *et al*., 2015; Sharma and Sharma, 2017; Wu *et al*., 2017). In plants, arbuscular mycorrhizas need carbohydrates and fatty acids of host plants to sustain fungal growth, and mycorrhizal symbiosis helps plants absorb water and nutrients (Wu *et al*., 2019). Oyewole *et al*. (2017) reported that inoculation with *Glomus deserticola* and *Gigaspora gigantea* exhibited inhibitive effects on charcoal rot disease of cowpea. Mycorrhizal cyclamen plants had higher resistance to Fusarium wilt and anthracnose disease (Maya and Matsubara, 2013). The inoculation with *Glomus sp.* MUCL 41833 strongly decreased leaf infection index of *Phytophthora infestans*, and also induced expressions of pathogenesis related genes (PR) *PR1* and *PR2* in potato, indicating systemic resistance appeared in mycorrhizal seedlings (Gallou *et al*., 2011).

In fact, many signal substances such as nitric oxide (NO), hydrogen peroxide (H_2O_2), salicylic acid (SA), and jasmonic acid (JA) are involved in system acquired resistance (SAR) formation by mycorrhizal induction and play an important role in increasing disease resistant.

Received: 09 May 2019. Received in revised form: 15 Aug 2019. Accepted: 01 Nov 2019. Published online: 22 Nov 2019.

Hypersensitive responses in plants are induced by pathogen infection accompanied with accumulation of NO and reactive oxygen species (ROS), which further induces the expression of defense genes and SAR establishment (Delledonne, 2005). In general, NO is increased in pathogen-infected plants (i.e., tobacco), and the NO peak is appear at 5 minutes after infecting by pathogen (Foissner *et al*., 2000). H_2O_2, a kind of ROS, plays an important role in defense gene expression, which can induce the expression of *PAL* (phenylalnine ammonialyase) and *CHS* (chalcone synthase) (Neill *et al*., 2002; Zhang *et al*., 2013). Calcium iron (Ca^{2+}), a common signal molecule in plants to respond pathogen infection, has the capacity of the early immune reaction in cells to resist pathogen (DeFalco *et al*., 2010). Calmodulin (CaM) is one of the Ca^{2+} receptor, and Ca/CaM activates many biochemical processes to enhance pathogen resistance (Kim *et al*., 2009). Exogenous application of SA helps pepper plants to resist blight by inducing the protein expression participating in defense systems (Chandrasekhar *et al*., 2017). The rice seedlings treated by exogenous methyl jasmonate owned the resistance for bacterial blight by up-regulating the expression of defense enzymes genes (Azami-Sardooei *et al*., 2010). These results demonstrate that many signalling substances are involved in the process of plant disease resistance, while it is not known how mycorrhiza-enhancing disease resistance of host plants is associated with changes of signalling substances and pathogen defense gene expression.

The present study tried to inoculate an AM fungus into trifoliate orange (*Poncirus trifoliata* L. Raf., a common rootstock of citrus used in Southeast Asia) and further illuminated the changes of signalling substance concentrations and pathogen defense gene expression after Xac infection.

Materials and Methods

Experimental design

The experiment was arranged in completely randomized blocked design with the inoculation with or without *Paraglomus occultum* (+AMF or –AMF) and the infection with or without *Xanthomonas axonopodis* pv. *citri* (+Xac or –Xac), in total of four treatments. The four treatments were expressed as +AMF+Xac, +AMF–Xac, –AMF+Xac, and –AMF–Xac, respectively. Each treatment had four replications.

Plant culture

The seed of trifoliate orange was sterilized by 70% ethanol solutions, cleaned by 10% KOH solutions to remove pectin, and germinated at sterilized (0.11 MPa, 121 °C, 1 h) sands. Three four-euphylla seedlings with same size were transplanted into 1.6-L plastic pots, in which 1.3 kg of sterilized mixture of soil and vermiculite (5:1, v/v) was supplied. The seedlings were grown in a glasshouse, where day/night temperature was 25/19 °C, average air humidity 85%, and photo flux density 721-967 μmol/m^2/s.

An AM fungus, *Paraglomus occultum* (Walker) Morton & Redecker, was provided by the Bank of Glomeromycota in China and propagated by white clover (*Trifolium repens*) under potted conditions. The mycorrhizal inoculums included sands, spores and infected root segments, whilst the spore density was 12 spores/g inoculum. *Xanthomonas axonopodis* pv. *citri* (Xac) was provided by the National Center for Citrus Improvement (Changsha), China, and further propagated based on the protocol described by Liu *et al*. (2012).

At transplanting, the seedlings were inoculated with 120 g mycorrhizal inoculums (approx. 1500 spores) per pot, and non-AMF treatment was applied with 120 g sterilized substrates plus 3 mL inoculums filtrate (25 μm filter) for similar microbial communities except spores of *Paraglomus occultum*. After 10 weeks of transplanting, the Xac was inoculated by needle puncturing method (Deng *et al*., 2010), and the fifth and sixth leaves on the top of seedlings were treated with 20 μL (10^8 CFU/mL) bacterial suspension. The Xac-treated wound was covered by filter paper (0.78 cm^3/piece). The bacterial suspension was replaced by sterilized distilled water in non-Xac treatment. After 5 days of Xac infection (water-soaked spot appeared), the seedlings were harvested.

Variable determinations

Before harvest, the plant height, stem diameter and leaf number were measured.

The 1-2 cm root segment was cleared by 10% KOH at 90 °C and stained with 0.05% typan blue in lactophenol solutions (Philips and Hayman, 1970). The root mycorrhizal colonization was expressed as the percentage in the colonized root lengths versus total observed root lengths. The soil hyphae length was determined according to the protocol of Bethlenfalvay and Ames (1987).

The 0.2 g root samples were homogenized with 0.1 mol/L phosphate buffer (pH 7.0) to assay the concentration of signal substrates, including SA, JA, NO, and CaM, as per the double antibody sandwich method. The measure was done according to user's handbook of kits which were produced by Shanghai Enzyme-linked Biotechnology Co., Ltd, China.

Root H_2O_2 was extracted by 10% trichloroacetic acid solutions and detected in the mixture of 1 mL supernatant, 1 mL potassium phosphate buffers, and 2 mL potassium iodide at 390 nm (Velikova *et al*., 2000).

Root RNA was extracted by the EASY Spin Plus RNA kit (RN38, Aidlab Biotechnologies CO. Ltd, China), and the reverse transcription was proceeded by the reverse transcription kit (RP047A, TAKARA). The qRT-PCR experiment was performed in the Bio-Rad CFX Connect Real-Time System (Bio-Rad Laboratories, Inc., US) with 10 μL system. The gene sequences used in the experiment were selected based on the RNA-seq data of trifoliate orange, and the primer sequence was shown in Table 1. Relative expression was calculated according to the method of Livak and Schmittgen (2001). Actin-7 was used as the reference gene.

Statistical analysis

Data (means ± SD, n = 4) were analyzed with the ANOVA (SAS, version 8.1), and the significantly difference between treatments was compared by the Duncan's multiple range test at 0.05 levels.

Table 1. The primer sequences of relevant genes involved in SA signaling in trifoliate orange

Gene name	Primer sequence(5'→3')	
	Forward primer	Reverse primer
PtPAL	TCTTACGGCGTTACCACTG	TGACTCTGTTCCGTTTCCA
PtEPS1	CAAGCGTGTAGCAACCAA	GCGTAAATGGACGGAGTG
PtPR4	GCCGCAGAAGGCAGTCCA	TCCAATGTGAGGGCAACT
Actin-7	CCGACCGTATGAGCAAGGAAA	TTCCTGTGGACAATGGATGGA

Results

Plant growth

As shown in Table 2, AMF colonization significant increased plant height and leaf number, but did not affect stem diameter, regardless of Xac infection or not. Xac treatment did not significantly alter plant growth performance, irrespective of AMF inoculation or not.

Mycorrhizal colonization and soil hyphal length

Root mycorrhizal colonization ranged from 26.9% under +AMF−Xac conditions to 27.2% under +AMF+Xac conditions, and soil hyphal length was 26.2 cm/g under +AMF+Xac conditions to 25.7 cm/g under +AMF−Xac conditions (Fig. 1). Xac infection did not alter root mycorrhizal colonization and soil hyphal length.

Root H_2O_2 concentrations

Under non-Xac infection conditions, AMF inoculation significantly decreased root H_2O_2 levels by 36%, whereas increased it by 74% in the present of Xac, compared with non-AMF treatment (Fig. 2). Compared with infection without Xac, Xac infection affected root H_2O_2 concentrations: decrease it by 27% in non-AMF plants and increase it by 97% in AMF plants.

Root NO concentrations

As shown in Fig. 3, both Xac infection and AMF inoculation influenced root NO concentrations. AMF treatment reduced root NO concentrations by 37% under non-Xac conditions but increased it by 65% under Xac infection condition. In addition, root NO concentrations were decreased by Xac infection in the absence of AMF.

Root CaM concentrations

AMF inoculation and Xac infection both significantly increased root CaM contents (Fig. 4). Root CaM concentrations were increased by mycorrhizal inoculation: 216% and 148% higher under non-Xac and Xac infection conditions, respectively. Xac infection also increased root CaM contents, and the increment was 88% higher in non-AMF plants and 47% higher in AMF plants.

Root JA concentrations

Root JA concentrations were ranked as +AMF−Xac > −AMF+Xac≈+AMF+Xac > −AMF−Xac in the decreased order (Fig. 5). Inoculation with AMF significantly increase root JA concentrations under non -Xac conditions. Xac infection significantly increased root JA concentrations under non-AMF conditions but significantly decreased root JA concentrations under +AMF conditions.

Table 2. The effect of *Paraglomus occultum* and *Xanthomonas axonopodis* pv. *citri* on plant growth of trifoliate orange seedlings

Treatments	Plant height (cm)	Stem diameter (mm)	Leaf number (#/plant)
-AMF-Xac	20.60±0.94b	2.25±0.07a	20±2b
+AMF-Xac	25.32±1.55a	2.29±0.14a	23±1a
-AMF+Xac	21.18±1.68b	2.24±0.08a	21±2b
+AMF+Xac	24.78±2.81a	2.27±0.19a	24±1a

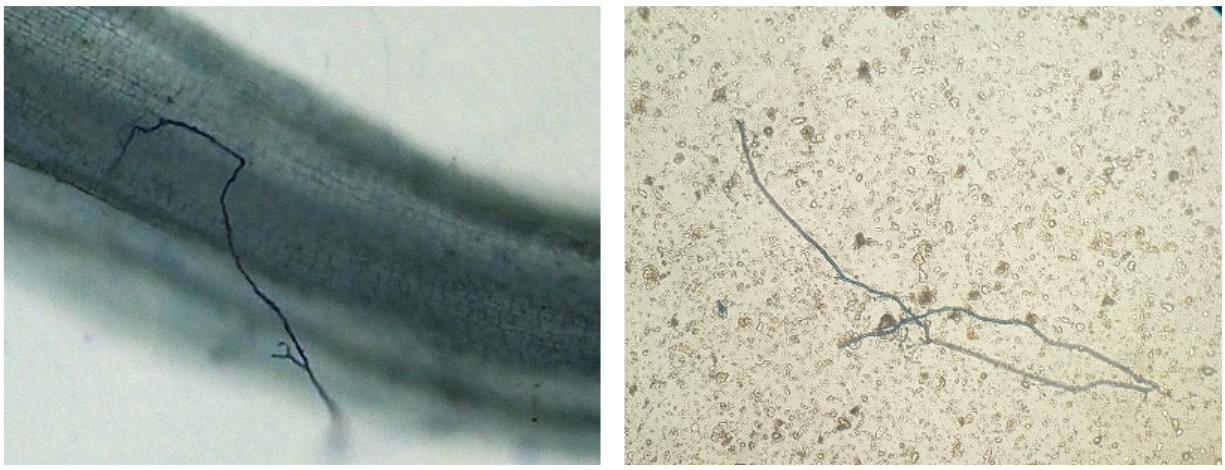

Fig. 1. The extraradical hyphae (left) and soil hyphae (right) of *Paraglomus occultum* in trifoliate orange seedlings

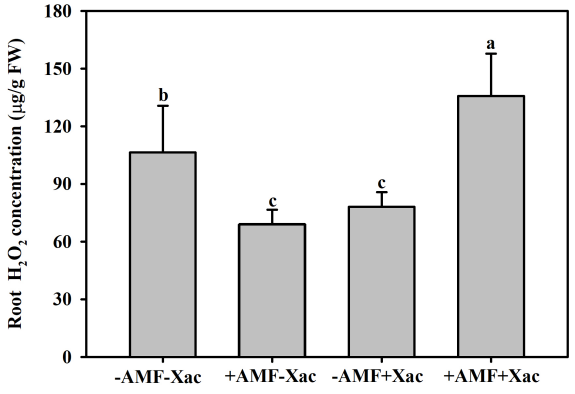

Fig. 2. The effect of *Paraglomus occultum* and *Xanthomonas axonopodis* pv. *citri* on root H_2O_2 concentrations in trifoliate orange seedlings. Data (means ± SD, n = 4) with different letter at the bar indicated the significant difference among treatments at 0.05 levels

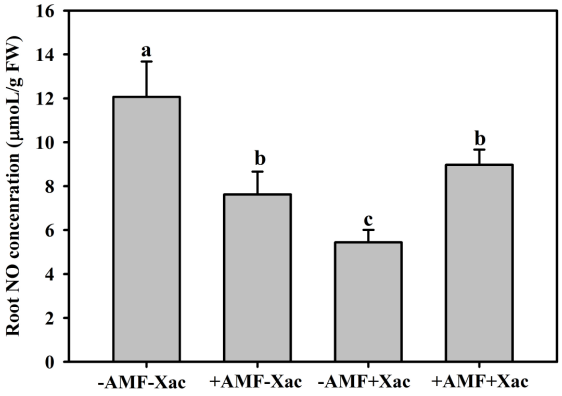

Fig. 3. The effect of *Paraglomus occultum* and *Xanthomonas axonopodis* pv. *citri* on root NO concentrations in trifoliate orange seedlings. Data (means ± SD, n = 4) with different letter at the bar indicated the difference was significant among treatments at 0.05 levels

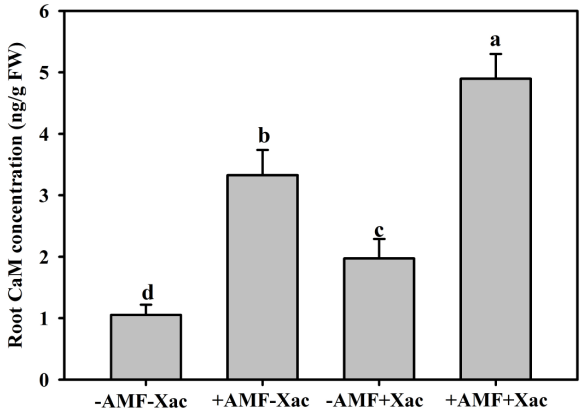

Fig. 4. The effect of *Paraglomus occultum* and *Xanthomonas axonopodis* pv. *citri* on root CaM concentrations in trifoliate orange seedlings. Data (means ± SD, n = 4) with different letter at the bar indicated the significant difference among treatments at 0.05 levels

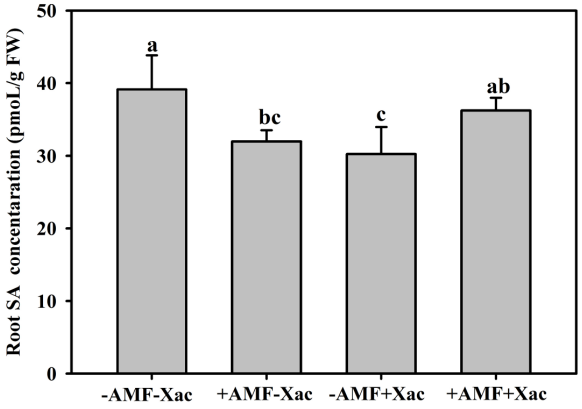

Fig. 5. The effect of *Paraglomus occultum* and *Xanthomonas axonopodis* pv. *citri* on root JA concentrations in trifoliate orange seedlings. Data (means ± SD, n = 4) with different letter at the bar indicated the significant difference among treatments at 0.05 levels

Root SA concentrations

AMF inoculation markedly decreased root SA concentrations by 18% in the case of non-Xac infection while increased root SA concentrations by 20% in the case of Xac infection (Fig. 6). In addition, Xac infection decreased SA concentrations in –AMF+Xac treatment, compared with –AMF–Xac treatment.

Relative expression of pathogen defense genes in roots

Xac inoculation significantly decreased the expression of *PtPAL* in roots regardless of inoculation with or without AMF, while AMF inoculation had no effect on *PtPAL* expression under –Xac or +Xac conditions (Fig. 7). AMF and Xac inoculation had significant effects on *PtEPS1* expression in roots. AMF inoculation significantly decreased *PtEPS1* expression in roots by 25% under –Xac conditions but increased it by 46% under +Xac conditions (Fig. 7). Xac infection also decreased root *PtEPS1* expression in the case of non-AMF inoculation. There were higher expression levels of *PtPR4* in +AMF treatment versus –AMF treatment, regardless of Xac infection or not (Fig. 7). Meanwhile, the *PtPR4* expression in roots was distinctly increased in +AMF+Xac treatment, as compared with +AMF–Xac treatment.

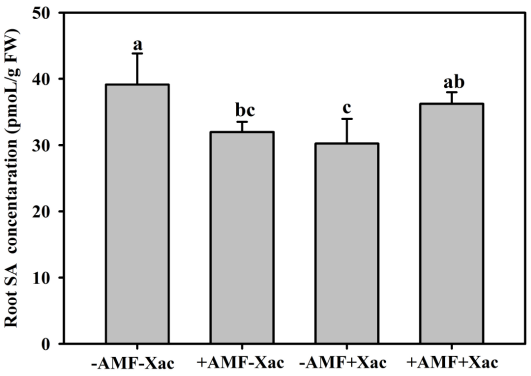

Fig. 6. The effect of *Paraglomus occultum* and *Xanthomonas axonopodis* pv. *citri* on root SA concentrations in trifoliate orange seedlings. Data (means ± SD, n = 4) with different letter at the bar indicated the significant difference among treatments at 0.05 levels

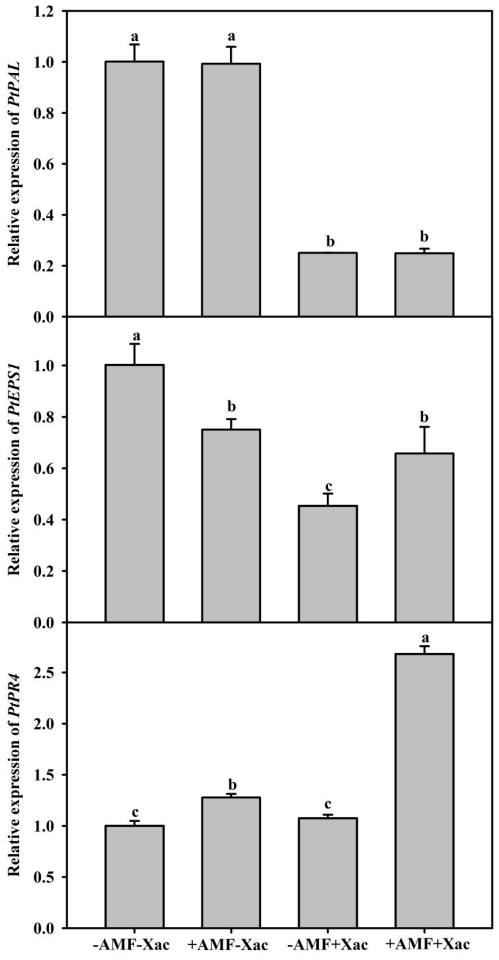

Fig. 7. The effect of *Paraglomus occultum* and *Xanthomonas axonopodis* pv. *citri* on relative expression of root *PtPAL*, *PtEPS1*, and *PtPR4* genes in trifoliate orange seedlings. Data (means ± SD, n = 3) with different letter at the bar indicated the significant difference among treatments at 0.05 levels

Discussion

The present study indicated a positive effect of AMF inoculation on plant growth of trifoliate orange, which also had been proved in various host plants such as *Metrosideros laurifolia* (Amie *et al*., 2019), maize (Ghorchiani *et al*., 2018), tea (Shao *et al*., 2018) and tomato (Vani *et al*., 2018). The Xac infection had no effect on plant growth of trifoliate orange and root mycorrhizal colonization, the possible reason of which was short-term time for the Xac infection (5 days), not enough to cause any responses of plant growth and mycorrhizas.

It is documented that H_2O_2 takes part in earliest defense responses to pathogen and also enhances the rigidity of structural proteins in cell walls to prevent pathogen infection (Lamb and Dixon, 1997; Grant and Loake, 2000). On the other hand, H_2O_2 can active expression of chitinases, peroxidases and lignin (Wu *et al*., 1997). Mohase *et al*. (2011) showed that there were higher H_2O_2 levels in resistant sunflower plants to resist rust. In our work, root H_2O_2 levels were the highest under +AMF+Xac treatment conditions, indicating that the trifoliate orange seedlings induced stronger resistance. In addition, mycorrhizal plants recorded significantly higher root H_2O_2 levels than non-mycorrhizal plants under +Xac infection conditions, meaning that SAR has been formed in the AMF seedlings, and AMF accelerates H_2O_2 accumulation to tolerate Xac infection. However, compared with –AMF–Xac treatment, the H_2O_2 levels were decreased in +AMF–Xac treatment, based on the fact of which non-Xac plants did not need such H_2O_2 response and mycorrhizas accelerate root H_2O_2 effluxes into rhizosphere (Zou *et al*., 2015).

In our work, AMF inoculation under –Xac conditions and Xac infection under non-mycorrhization collectively decreased root NO concentrations, the reason of which may be the fact that sole microorganism inoculation induces lower root NO levels, or the NO peak is missed (Zhang *et al*., 2017a). Nevertheless, under Xac infection, AMF inoculation significantly increased root NO levels, as compared with non-AMF treatment, indicating that mycorrhizas stimulate root NO accumulation under Xac infection. More NO may be used to modify proteins to perform various functions in defense responses (Lindermayr *et al*., 2005).

Zhang *et al*. (2010) found the CaM binding protein CBP60g induced the expression of SA synthetic gene *ICS1* and further activated SA biosynthesis and defense response. Hence, CaM is pivotal for activating downstream defense response. It is known that AMF inoculation could enhance the expression of CaM in host plants exposed to abiotic stress, such as drought stress (Huang *et al*., 2014). The mycorrhizal soybean showed higher CaM concentrations to capture signals (Lorella *et al*., 2007). In this work, AMF inoculation and Xac infection collectively increased root CaM concentrations of trifoliate orange, indicating that the CaM-signal pathway had been activated by Xac infection and AMF inoculation to reinforce the effect. AMF plants represented higher root CaM levels than non-AMF plants under Xac or non-Xac infection conditions, implies that mycorrhizas further strengthen the CaM-signal pathway to respond Xac infection by inducing more 'signal receiver'.

There are SA and JA pathways to transfer signals in plants, and SA and JA pathways exhibit synergistic effects at low concentrations but antagonistic at high concentrations (Spoel and Dong, 2008). Compared with −AMF−Xac treatment, +AMF−Xac treatment significantly increased root JA contents and the expression of *PtPR4*, while decreased root SA contents and relative expression of *PtEPS1*, indicating that without Xac infection, mycorrhizal plants depend JA and *PtPR4* to enhance resistance. The result is consistent with previous study in which AMF inoculation primed disease resistance of tomato to resist early blight disease (Song *et al*., 2015). The expression of *PtPR4* was not changed by Xac infection in non-mycorrhizal plants but significantly increased in pro-inoculated AMF plants, indicating that primed mycorrhizal plants are easier to deal with the Xac infection.

In our previous studies, JA and SA concentrations were significantly increased when suberification was formed in the Xac-infected site (Zhang *et al*., 2017b, 2019). However, in this work, only the JA content was increased when water stain appeared in Xac-treated plants. Hence, we speculated that SA and JA play different roles during Xac infection. Nair *et al*. (2015) inoculated AMF and *Fusarium oxysporum* on tomato and found that JA took part in the expression of resistance and SA induced defense responses. In early stage of pathogen infection, JA could thicken cell wall, induce defense substrates (proteinase, PAL and LOX) synthesis, and oxygen burst to enhance resistance (Azami-Sardooei *et al*., 2010). However, SA may work in prolonged response ageing. More works in JA and SA responses need to be done under mycorrhization and Xac infection.

Acknowledgements

This study was supported by the Plan in Scientific and Technological Innovation Team of Outstanding Young Scientists, Hubei Provincial Department of Education (T201604).

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

References

Amir H, Cavaloc Y, Laurent A (2019). Arbuscular mycorrhizal fungi and sewage sludge enhance growth and adaptation of *Metrosideros laurifolia* on ultramafic soil in New Caledonia: A field experiment. Science of the Total Environment 651(1):334-343.

Azami-Sardooei Z, Franca SC, De Vlesschauwer D, Höfte M (2010). Riboflavin induces resistance against *Botrytis cinerea* in bean, but not in tomato, by priming for a hydrogen peroxide-fueled resistance response. Physiological and Molecular Plant Pathology 75(1-2):23-29.

Bethlenfalvay GJ, Ames RN (1987). Comparison of two methods for quantifying extraradical mycelium of vesicular arbuscular mycorrhizal fungi. Soil Science Society of America Journal 51(3):834-837.

Chandrasekhar B, Umesha S, Naveen Kumar HN (2017). Proteominc analysis of salicylic acid enhanced disease resistance in bacterial wilt affected chili (*Capsicum annuum*) crop. Physiological and Molecular Plant Pathology 98:85-96.

Das AK (2003). Citrus canker - A review. Journal of Applied Horticulture 5(1):52-60.

DeFalco TA, Bender KW, Snedden WA (2010). Breaking the code: Ca^{2+} sensors in plant signaling. Biochemical Journal 425(1):27-40.

Delledonne M (2005). NO news is good news for plant. Current Opinion in Plant Biology 8(4):390-396.

Deng ZN, Xu L, Long GY, Liu LP, Fang FF, Shu GP (2010). Screening citrus genotypes for resistance to canker disease (*Xanthomonas axonopodis* pv. *citri*). Plant Breeding 129(3):341-345.

Foissner I, Wendehenne D, Langebartels C, Durner J (2000). *In vivo* imaging of an elicitor-induced nitric oxide burst in tobacco. Plant Journal 23(6):817-824.

Gallou A, Mosquera HPL, Cranenbrouck S, Suárez JP, Declerck S (2017). Mycorrhiza induced resistance in potato plantlets challenged by *Phytophthora infestans*. Physiological and Molecular Plant Pathology 76(1):20-26.

Ghorchiani M, Etesami H, Alikhani HA (2018). Improvement of growth and yield of maize under water stress by co-inoculating an arbuscular mycorrhizal fungus and a plant growth promoting rhizobacterium together with phosphate fertilizers. Agriculture Ecosystems and Environment 258:59-70.

Grant, JJ, Loake, GJ (2000). Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. Plant Physiology 124(1):21-29.

Huang YM, Srivastava AK, Zou YN, Ni QD, Han Y, Wu QS (2014). Mycorrhizal-induced calmodulin mediated changes in antioxidant enzymes and growth response of drought-stressed trifoliate orange. Frontiers in Microbiology 5(682):1-7.

Kim MC, Chung WS, Yun DJ, Cho MJ (2009). Calcium and calmodulin-mediated regulation of gene expression in plants. Molecular Plant 2(1):13-21.

Lamb C, Dixon RA (1997). The oxidative burst in plant disease resistance. Annual Review of Plant Physiology and Plant Molecular Biology 48(1):251-275.

Lindermayr J, Saalbach G, Durner J (2005). Proteomic identification of S-nitrisylated proteins in *Arabidopsis*. Plant Physiology 137(3):921-930.

Liu JY, Maldonado-Mendoza L, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ (2007). Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. Plant Journal 50(3):529-544.

Liu LP, Deng ZN, Qu JW, Yan JW, Catara V, Li DZ, Long GY, Li N (2012). Construction of EGFP-labeling system for visualizing the infection process of *Xanthomonas axonopodis* pv. *citriinplanta*. Current Microbiology 65(3):304-312.

Livak LJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and $2^{-\Delta\Delta Ct}$ method. Methods 25(4):402-408.

Lorella N, Moscatiello R, Genre A, Novero M, Bonfante P, Mariani P (2007). The arbuscular mycorrhizal fungus *Glomus intraradices* induces intracellular calcium changes in soybean cells. Caryologia 60(1-2):137-140.

Maya MA, Matsubara YI (2013). Tolerance to Fusarium wilt and anthracnose diseases and changes of antioxidative activity in mycorrhizal cyclamen. Crop Protection 47(5):41-48.

Mohase L, der Westhuizen AJ, Pretorius ZA (2011). Involvement of reactive oxygen species generating enzymes and hydrogen peroxide in the rust resistance response of sunflower (*Helianthus annuus* L.). South African Journal of Plant and Soil 28(1):64-68.

Nair A, Kolet SP, Thilasiram HV, Bhargava S (2015). Systemic jasmonic acid modulation in mycorrhizal tomato plants and its role in induced resistance against *Alternaria alternate*. Plant Biology 17(3):625-631.

Nair A, Kolet SP, Thulasiram HV, Bhargava S (2105). Role of methyl jasmonate in the expression of mycorrhizal induced resistance against *Fusarium oxysporum* in tomato plants. Physiological and Molecular Plant Pathology 92:139-145.

Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT (2002). Hydrogen peroxide and nitric oxide as signalling molecules in plants. Journal of Experimental Botany 53(372):1237-1247.

Oyewole BO, Olawuyi OJ, Odebode AC, Abiala MA (2017). Influence of arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea. Biotechnology Reports 14:8-15.

Parniske M (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nature Reviews Microbiology 6(10):763-775.

Phillips JM, Hayman DS (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(1):158-161.

Romero-Puertas MC, Perazzolli M, Zago E, Delledonne M (2004). Nitric oxide signaling functions in plant interaction. Cellular Microbiology 6(9):795-803.

Shao YD, Zhang DJ, Hu XC, Wu QS, Jiang CJ, Xia TJ, Gao XB, Kuča K (2018). Mycorrhiza-induced changes in root growth and nutrient absorption of tea plants. Plant Soil and Environment 64(6):283-289.

Sharma IP, Sharma AK (2017). Co-inoculation of tomato with an arbuscular mycorrhizal fungus improves plant immunity and reduces root-knot nematode. Rhizosphere 4:25-28.

Song YY, Chen DM, Lu K, Sun ZX, Zeng R (2015). Enhances tomato disease resistance primed by arbuscular mycorrhizal fungus. Frontiers in Plant Science 6(786):1-13.

Spoel SH, Dong X (2008). Making sense of hormone crosstalk during plant immune response. Cell Host Microbe 3(6):348-351.

Vani MS, Hindumathi A, Reddy BN (2018). Beneficial effect of arbuscular mycorrhizal fungus, *Glomus fasciculatum*, on plant growth and nutrient uptake in tomato. Indian Phytopathology 71(1):115-122.

Velikova V, Yordanov I, Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protection of exogenous polyamines. Plant Science 15(1):59-66.

Wu G, Shortt BJ, Lawrence EB, Leo J, Fitzsimmons KC, Levine EB, Raskin I, Shah DM (1997). Activation of host defense mechanisms by elevated production of H_2O_2 in transgenic plants. Plant Physiology 115(2):427-435.

Wu QS, He JD, Srivastava AK, Zou YN, Kuca K (2019). Mycorrhizas enhance drought tolerance of citrus by altering root fatty acid compositions and their saturation levels. Tree Physiology 39(7):1149-1158.

Wu QS, Sun P, Srivastava AK (2017). AMF diversity in citrus rhizosphere. Indian Journal of Agricultural Sciences 87(5):653-656.

Zhang RQ, Zhu HH, Zhao HQ, Yao Q (2013). Arbuscular mycorrhizal fungal inoculation increases phenolic synthesis in clover roots via hydrogen peroxide, salicylic acid and nitric oxide signaling pathways. Journal of Plant Physiology 170(1):74-79.

Zhang Y, Xu S, Ding P, Wang DM, Cheng YT, He J, ... Zhang YL (2010). Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proceedings of the National Academy of Sciences 107(42):18220-18225.

Zhang YC, Liu CY, Wu QS (2017a). Mycorrhizal and common mycorrhizal network regulated the production of signal substances in trifoliate orange (*Poncirus trifoliata*). Notulae Botanicae Horti Agrobotanici Cluj-Napoca 45(1):43-49.

Zhang YC, Liu LP, Zou YN, Liu CY, Wu QS (2017b). Response of signal substances to canker in trifoliate orange roots through mycorrhizal hyphal bridge. Mycosystema 36(7):1028-1036 (in Chinese with an English abstract).

Zhang YC, Zou YN, Liu LP, Wu QS (2019). Common mycorrhizal networks activated salicylic acid defense response of trifoliate orange (*Poncirus trifoliata*). Journal of Integrative Plant Biology 61(10):1099-1111.

Zou YN, Huang YM, Wu QS, He XH (2015). Mycorrhiza-induced lower oxidative burst is related with higher antioxidant enzyme activities, net H_2O_2 effluxes, and Ca^{2+} influxes in trifoliate orange roots under drought stress. Mycorrhiza 25(2):143-152.