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ORIGINAL ARTICLE

Mycorrhiza-induced plant defence responses in trifoliate orange infected by *Phytophthora parasitica*

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Abstract

Arbuscular mycorrhizal fungi (AMF) reduce disease incidence of host plants through the competition of carbon sources and direct inhibition of pathogens, as well as through induction of biochemical and molecular responses. However, it is not known whether AMF enhance the resistance to *Phytophthora parasitica*-induced root rot in citrus and what the underlying mechanisms are. This study was carried out to analyze roles of *Funneliformis mosseae* (a mycorrhizal fungus) in plant defence responses of *Poncirus trifoliata* infected by *P. parasitica*. A week after the pathogen infection, mycorrhizal seedlings possessed higher expression of root mitogen-activated protein kinase 3 (*PtMAPK3*) regardless of *P. parasitica* infection. *F. mosseae* induced higher root salicylic acid (SA) concentrations, accompanied with up-regulation of SA synthesis genes (*PtPAL1* and *PtEPS1*), regardless of being infected with *P. parasitica* or not. Jasmonic acid (JA) synthesis genes were down-regulated by mycorrhization in the absence of *P. parasitica* and up-regulated (except for *PtAOC*) by mycorrhization under *P. parasitica* infection. Moreover, *F. mosseae* stimulated higher expression of pathogenesis-related protein gene 1 (*PtPR1*), *PtPR4*, and *PtPR5*, especially under *P. parasitica* infection. *F. mosseae* inoculation increased levels of root lignin, calmodulin, and total soluble phenol and activities of root chitinase, phenylalanine ammonialyase, and β-1,3-glucanase, and decreased concentrations of root nitric oxide with or without *P. parasitica* infection. These results implied that *F. mosseae* elicited MAPKs cascades as well as SA- and calmodulin-mediated signal pathways to activate disease-defence genes, proteins, and compounds to early-warn *P. parasitica* infection for enhancing tolerance of root rot in trifoliate orange.

Keywords Arbuscular mycorrhiza · Citrus · Root rot · Salicylic acid

Introduction

Citrus is one of the widely planted economic fruit trees, of which China ranks first in the world in terms of planted area and production in citrus industry (He et al. 2020a). Citrus trees are vulnerable to various diseases in the field, including citrus root rot. Earlier studies had shown that citrus root rot was triggered mainly by *Phytophthora parasitica* in China (Zhu et al. 1993). In the early stage of infection, *Phytophthora parasitica* formed the haustoria, modified plant metabolic activities, and thus inhibited host defenses (O'Connell and Panstruga 2006). Pesticides are heavily used to mitigate the crop losses caused by *P. parasitica* which seriously damages the environment.

Symbiotic root endophytes can improve plant growth and enhance plant-disease defences (Gao et al. 2020; Yang et al. 2021). In particular, arbuscular mycorrhizal fungi (AMF), from the phylum Glomeromycota , build symbiotic associations with approximately 72% of terrestrial plants for increasing plant's capacity to absorb nutrients beyond the nutrient depletion zone (Ferrol et al. 2019; Wu et al. 2019; Xie et al. 2020). Additionally, AMF improve the tolerance of plants in response to abiotic and biotic stress (Sarfir 1968; Xie et al. 2019; He et al. 2020b; Zhang et al. 2019, 2020; Zou et al. 2021). Inoculation with *Funneliformis mosseae* (formerly *Glomus mosseae*) significantly inhibited *Pyrenochaeta* infection and decreased the morbidity of red rot in *Allium cepa* (Sarfir 1968). *Funneliformis mosseae* and *Rhizophagus irregularis* collectively mitigated symptoms

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of *Aphanomyces euteiches*-induced root rot in pea (Slezack et al. 1999). Giovannetti et al. (1991) found that *Glomus monosporum* considerably reduced the formation of chlamydospores of *Thielaviopsis basicola* and the infection process in tobacco. As a result, it seems that mycorrhizalized plants have a high capacity to resist root rot, while the underlying mechanisms are not fully known.

Protein phosphorylation and de-phosphorylation processes are regulatory mechanisms involved in plant pathological processes (Pitzschke et al. 2009). Among them, protein phosphorylation is catalyzed by protein kinases including mitogen-activated protein kinases (MAPKs) (Meng and Zhang 2013). MAPK cascades have a dominant role in the amplification and transmission of signals (Aguilar et al. 2017) and thus help plants to resist pathogenic fungi, bacteria, nematodes, and insects (Wang et al. 2010).

Salicylic acid (SA) is known to be the primary signal in the regulation of plant immune responses (Chen et al. 2018). SA combines with SA-binding proteins (SABPs) to form SA–SABPs complexes, which can transfer the infection signal to trigger anaphylactic reactions (Alvarez 2000). Also, activation and interaction with transcription factors, such as non-expresser of pathogenesis-related gene 1 (NPR1), TGACG-binding factor (TGA), and WRKY (DNA binding transcription factors with highly conserved WRKYGDK domain), induced by the signal stimulus, increase the expression of PRs (Yu et al. 2001). On the other hand, jasmonic acid (JA) participates in the AMF-mediated resistance, while SA usually inhibits the biosynthesis of JA and JA-mediated defence responses (Robert-Seilaniantz et al. 2011). Nitric oxide (NO) stimulates SA accumulations, while SA induces NO production. It is not clear whether these signal responses are involved in AMF-induced tolerance against root rot.

The present work dually inoculated *Funneliformis mosseae* (an AM fungus) and *P. parasitica* (a root rot pathogen) on trifoliate orange (*Poncirus trifoliata*), and determined concentrations of root defence compounds, activities of root defence proteins, and relative quantities of root defence-related genes, to study the defence responses induced by AMF in trifoliate orange.

Materials and methods

Experimental design and microbial inoculations

The experiment consisted of two factors in a completely randomized blocked arrangement: the inoculation with or without *F. mosseae* and the infection with or without *P. parasitica* (Fig. 1). Each treatment was replicated five times, with three seedlings in each pot, resulting in a total of 20 pots (60 seedlings).

The AM fungal strain, *F. mosseae* (Nicol. & Gerd.) Schüßler and Walker, was propagated using white clover in pots, and the mycorrhizal fungal inoculum contained spores and the fungi-colonized root segments.

Three trifoliate orange seedlings having five leaves and without mycorrhization were transplanted into a 1.6-L pot. The pot contained 1.5 kg autoclaved mixture with soil and sand (5: 1, v/v) and 90 g of mycorrhizal inoculum. The treatment without AMF received an equivalent amount of autoclaved inocula plus 2 mL filtrate (25 μm filter) of mycorrhizal inocula.

Fourteen weeks after the mycorrhizal fungal inoculation, the pathogenic fungus *P. parasitica* was applied. *Phytophthora parasitica* was from the Citrus Research Institute, SWU/CAAS, Chongqing, China. The infected method of *P. parasitica* was followed with Cheng et al. (2020) in detail. A plug of sterile potato dextrose agar was placed onto the wound of root necks as the non-*P. parasitica*-infected controls. Both the *P. parasitica*-infected and non-*P. parasitica*-infected plants were covered with the moist sterile absorbent cotton. One week after the pathogen inoculation, the experiment ended and the treated plants were collected and divided into shoots and roots. Parts of roots were collected for the analysis of root AMF colonization rate, and the other roots were stored at – 80 °C after freezing in liquid nitrogen for the analysis of concentrations of SA, JA, NO, and calmodulin (CaM), activities of chitinase, β-1,3-glucanase, and phenylalanine ammonia-lyase (PAL), and expression of disease-related genes.

All the plants were placed in a greenhouse from April 8 to July 22, 2017, where the photon flux density ranged from 721 to 967 μmol/m^2/s, with 25 °C/19 °C average day/night temperature and 68% relative air humidity.

Determinations of root mycorrhizal colonization

Root arbuscular mycorrhizas (10 root segments per plant and 5 plants per treatment) were stained with 0.05% trypan blue in lactoglycerol according to the protocol of Phillips and Hayman (1970). The microscopic observation was accorded to a fourfold objective lens multiplied by a 25-fold eyepiece. The diameter of one field was 300 scales. The extent of root mycorrhizal fungal colonization was calculated by He et al. (2020b).

Determinations of signal substances concentrations

The 0.2 g roots were ground in 1 mM phosphate buffers (pH 7.0) to extract NO, SA, and JA and homogenized in 0.6% NaCl solutions to extract CaM. The concentration of signal substances in roots was measured by double antibody sandwich-ELISA kits as per the user handbook (Shanghai Enzyme-linked Biotechnology Co., Ltd, Shanghai, China),

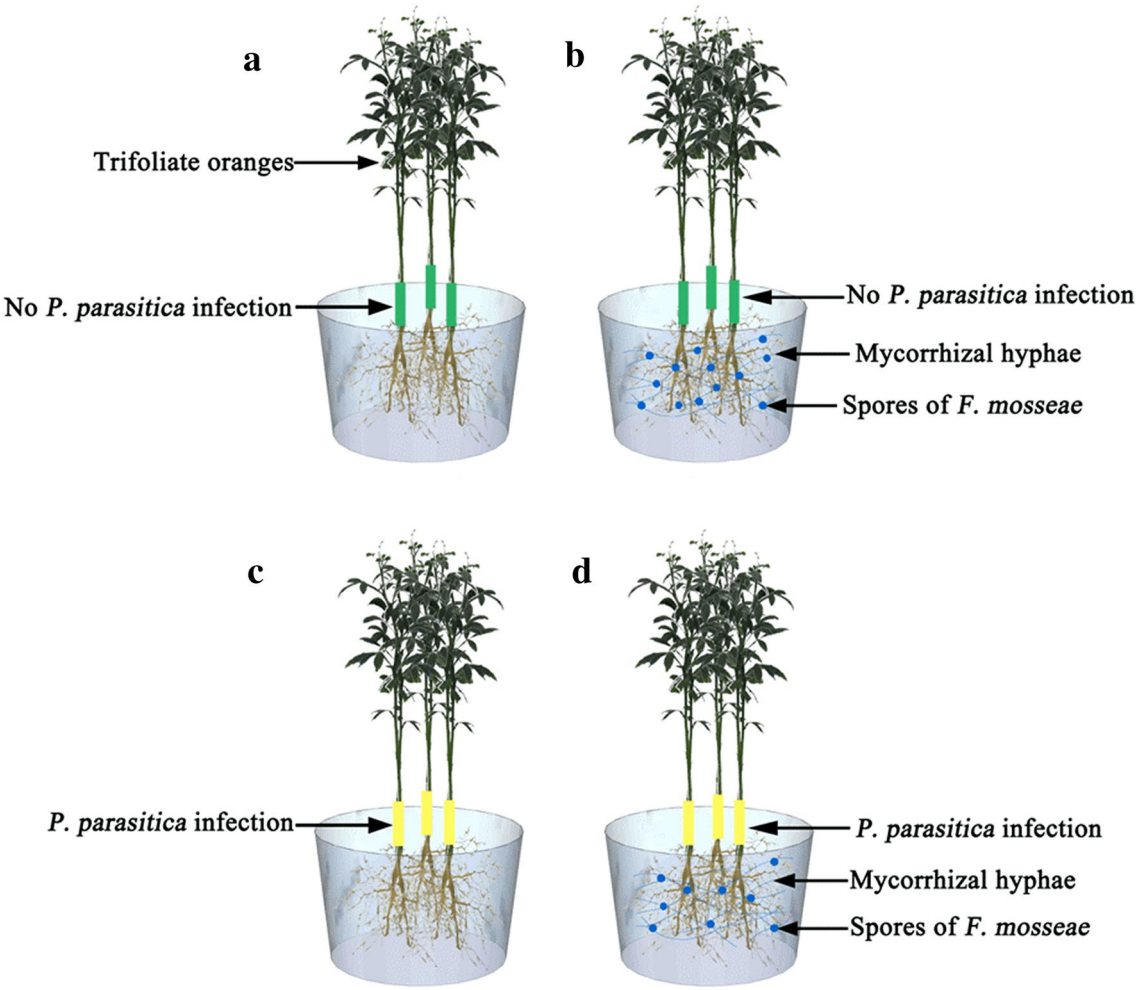

Fig. 1 The schematic diagram of the experimental design. **a** − *F.m*− *P.p* treatment; **b** + *F.m*− *P.p* treatment; **c** − *F.m* + *P.p* treatment; **d** + *F.m* + *P.p* treatment. *F.m* inoculation with *Funneliformis mosseae*, *P.p* infection by *Phytophthora parasitica*

along with the purified antibody of plant SA, JA, NO, and CaM as solid-phase antibody, horse radish peroxidase-labeled antibody, and the substrate 3,3′,5,5′-tetramethyl benzidine. The absorbance at 450 nm was determined by a Microplate Reader. All the determinations of the substances were repeated five times.

Determinations of disease-related compounds and proteins

Root total soluble phenol (TSP) and lignin concentrations were determined by the method of Kofalvi and Nassuth (1995) and Cahill and Mccomb (1992), respectively. Root β-1,3-glucanase and chitinase activities were determined by the protocol of Hu et al. (2017). Root PAL activity was carried out using the method described by Kofalvi and Nassuth (1995), where a unit of the enzyme activity was defined as the enzyme amount at 290 nm for a change of 0.01 unit of absorbance value per hour. All the above assays were replicated five times.

Relative expression of disease-related genes

We weighed 0.1 g of root samples, ground them in liquid nitrogen, and used an EASY spin Plus plant RNA kit to extract root total RNA. Then, 7 μL of RNA was reverse-transcribed into cDNA using a PrimeScript™ RT reagent kit with the gDNA eraser (Takara). qRT-PCR amplifications were conducted in a CFX96 Real-Time PCR Detection System (BIO-RAD) with the following composition: 3.5 μL sterile water, 0.5 μL cDNA, 5 μL SYBR GREEN PCR Master Mix (Applied Biosystem), 0.5 μL of 0.1 μM forward primer, and 0.5 μL of 0.1 μM reverse primer. These primers and gene ID for the selected genes, including pathogenesis-related (PR) genes (*PtPR1*, *PtPR4*, and *PtPR5*), phenylalanine ammonialyase 1 gene (*PtPAL1*), enhance

enolpyruvylshikimate phosphate synthase 1 gene (*PtEPS1*), lipoxygenase gene (*PtLOX*), allene oxide synthase gene (*PtAOS*), allene oxide cyclase gene (*PtAOC*), and mitogen-activated protein kinase 3 gene (*PtMPK3*) were designed as per the *Citrus sinensis* database (Table 1). The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Kenneth and Schmittgen 2001) in which β-actin acted as the control.

Statistical analysis

Data were subjected to the analysis of variance (SAS, v8.1), and significant differences between treatments were performed by the Duncan's multiple range tests at the 0.05 level.

Results

Root mycorrhizal colonization

Root mycorrhizal colonization varied from 26.4 to 33.0%, while *P. parasitica* infection significantly inhibited root AMF colonization degree by 20%, compared with non-*P. parasitica*-infected mycorrhizal seedlings (Table 2).

Concentrations and activities of root defensive compounds

Root SA in mycorrhizal seedlings showed 33% higher concentrations under non-*P. parasitica* infection and 55% higher under *P. parasitica* infection, respectively, relative to non-mycorrhizal plants (Table 2). Compared with the non-AMF treatments, AMF inoculation distinctly reduced root JA concentrations of trifoliate orange by 43% under non-*P. parasitica* infection and 36% under *P. parasitica* infection. Compared with the non-AMF treatments, mycorrhizal inoculation significantly reduced root NO concentrations of trifoliate orange, whether infected with *P. parasitica* or not: 19% lower without *P. parasitica* infection and 11% lower with *P. parasitica* infection, respectively. In contrast, mycorrhizal plants showed significantly higher root CaM concentrations than control plants by 17% and 27% under non-*P. parasitica* infection and *P. parasitica* infection, respectively. Additionally, AMF inoculation induced significantly higher root TSP and lignin concentrations than non-AMF seedlings: 19% and 10% higher without *P. parasitica* infection and 28% and 31% higher with *P. parasitica* infection, respectively. Mycorrhizal colonization significantly increased root β-1,3-glucanase, chitinase, and PAL activities by 13%, 65%, and 37% without *P. parasitica* infection and by 13%, 61%, and 52% with *P. parasitica* infection, respectively.

Expression of root disease-related genes

In the non-AMF-colonized plants, the *PtPR4*, *PtLOX*, and *PtAOS* expression was up-regulated by *P. parasitica* infection, whereas the *PtPAL1* expression was down-regulated by *P. parasitica* infection (Fig. 2). In the mycorrhizal plants, *P. parasitica* infection up-regulated *PtPR1*, *PtPR4*, *PtPR5*, *PtPAL1*, and *PtAOS* gene expression levels, whereas down-regulated *PtMAPK3* and *PtAOC* gene expression levels. Compared with non-AMF-colonized seedlings, AMF-colonized seedlings possessed 1182% and 240% higher expression levels of root *PtMAPK3* in the absence and presence of *P. parasitica* infection, respectively. Compared with the non-AMF-colonized treatment, AMF treatment notably increased the expression of root *PtPR1*, *PtPR4*, and *PtPR5* by 57%, 80% and 25% under the condition of non-*P. parasitica* infection, and 523%, 297% and 277% under the condition of *P. parasitica* infection. In contrast with the non-AMF-inoculated treatment, AMF-inoculated treatment notably increased the expression of root *PtPAL1* and *PtEPS1* by 107% and 210%, respectively, in trifoliate orange without *P. parasitica* infection. AMF inoculation also significantly promoted the expression of root *PtPAL1* and *PtEPS1* by 474% and 436% under *P. parasitica* infection, respectively, relative to non-mycorrhizal seedlings. Under non-*P. parasitica*

Table 1 The specific primers of relevant genes designed for real time quantitative PCR amplification

Gene name	Gene ID	Sequence (5′→3′)-forward	Sequence (5′→3′)-reverse
β-actin	*Cs1g05000*	CCGACCGTATGAGCAAGGAAA	TTCCTGTGGACAATGGATGGA
PtPAL1	*Cs6g11940*	TCTTACGGCGTTACCACTG	TGACTCTGTTCCGTTTCCA
PtEPS1	*Cs2g30570*	CAAGCGTGTAGCAACCAA	GCGTAAATGGACGGAGTG
PtPR1	*Cs4g02980*	GCGAGTTACGCACAGCAATA	GACTTCTTCTCCGAAATCCAC
PtPR4	*Cs8g13680*	GCCGCAGAAGGCAGTCCA	TCCAATGTGAGGGCAACT
PtPR5	*Cs3g24410*	TGATAGCGTTGTTGATGGGTT	TCCGATGGGCAGGTAAGC
PtLOX	*Cs3g13930*	GCATCCTTTCTTGATCGGTTTC	GGCAGGCTCGCCATG
PtAOS	*Cs3g24230*	ATCAAACGGCGGCAAAGTG	GTATTGTAACGCTACGGGTGG
PtAOC	*Cs6g18900*	AGATCGTGGCAGTCCAGCTT	GCTAAAAGGGACAAGATCACCAA
PtMPK3	*Cs8g17360*	GGTCCAAATGTCGCAGAA	ATACGGAGACGAGTGAGC

The gene ID was blast from the *Citrus sinensis* database

Table 2 Effects of *Funneliformis mosseae* inoculation and *Phytophthora parasitica* infection on concentrations of root defence compounds and activities of root defence proteins in *Poncirus trifoliata*

Treatments	Mycorrhizal colonization (%)	Concentrations of defence compounds						Activities of defence proteins (U/g FW)		
		SA (pmol/g FW)	JA (pmol/g FW)	NO (μmol/g FW)	CaM (μg/g FW)	TSP (μg/g FW)	Lignin (mg/g FW)	β-1,3-Glucanase	Chitinase	PAL
− *F.m*− *P.p*	0 ± 0c	81.92 ± 2.43c	64.96 ± 1.70a	38.75 ± 1.63c	6.98 ± 0.19b	41.49 ± 2.58b	4.11 ± 0.34b	6.74 ± 0.28b	1.28 ± 0.09c	2.09 ± 1.17c
+ *F.m*− *P.p*	33.0 ± 2.7a	108.79 ± 4.02a	37.11 ± 1.70c	31.23 ± 1.76d	8.17 ± 0.10a	49.40 ± 3.94a	4.52 ± 0.03a	7.64 ± 0.28a	2.11 ± 0.13a	2.86 ± 0.22a
− *F.m*+ *P.p*	0 ± 0c	67.69 ± 3.27d	51.27 ± 1.77b	64.40 ± 1.91a	5.68 ± 0.47c	36.84 ± 0.18c	3.29 ± 0.19c	6.20 ± 0.25c	1.13 ± 0.03d	1.55 ± 0.10d
+ *F.m*+ *P.p*	26.4 ± 1.6b	104.66 ± 1.09b	32.75 ± 1.65 cd	57.33 ± 1.42b	7.21 ± 0.16b	47.37 ± 2.87a	4.32 ± 0.25ab	7.03 ± 0.17b	1.82 ± 0.11b	2.35 ± 0.19b

Data (means ± SD, $n = 5$) followed by different letters in a column indicate significant differences ($p < 0.05$) between treatments

CaM calmodulin, *F.m* inoculation with *Funneliformis mosseae*, *JA* jasmonic acid, *NO* nitric oxide, *PAL* phenylalanine ammonialyase, *P.p* infection by *Phytophthora parasitica*, *SA* salicylic acid, and *TSP* total soluble phenol

infectious conditions, mycorrhizal inoculation reduced the expression of root *PtLOX* by 34%, in comparison with the non-mycorrhizal treatment. Apart from root *PtAOC*, which was down-regulated by 22%, the expression of the other two JA synthesis genes (*PtLOX* and *PtAOS*) was, respectively, increased by 56% and 23% under *P. parasitica* infection in mycorrhizal seedlings, compared with non-mycorrhizal seedlings.

Discussion

A week after the pathogen infection, there were relatively less visible decay areas in the root neck of AMF-colonized trifoliate orange plants than that of non-AMF-colonized plants, indicating the enhancement in tolerating root rot in mycorrhizal plants. The present work also showed that the pathogen infection with *P. parasitica* significantly reduced the root *F. mosseae*-colonized rate, indicating that *P. parasitica* had a suppressive role in mycorrhizal development. Similar result was reported by Ballhorn et al. (2014), who found that the infection of *Colletotrichum gloeosporioides* on leaves of soybean significantly reduced the colonization rate of AMF. Such inhibition might be the competition of carbon sources and infection sites between pathogens and beneficial endophytic fungi (Vos et al. 2014), thus leading to the decrease of mycorrhizal colonization rate.

Asai et al. (2002) found that the MAPKKK1-MAPKK4/5-MAPK3/6 cascade reaction chains were activated by flagellin which induced the expression of downstream WRKY22/WRKY29, and eventually activated the expression of relevant defence genes to enhance disease resistance. In the present study, mycorrhizal plants exhibited significantly higher expression of root *PtMAPK3* than non-mycorrhizal plants regardless of *P. parasitica* infection, and AMF- and *P. parasitica*-infected plants presented the highest expression level. It seemed that AMF had stimulated effect on *PtMAPK3* expression. Since SA generally restrains JA synthesis by up-regulating expression of *WRKY* transcription factors (*WRKY62* and *WRKY70*), MAPK cascades keep in close contact with SA-mediated signal pathways (Dóczi et al. 2007). Hence, it was assumed from this study that MAPK cascades cross-talked with SA-mediated signal pathways by regulating expression of *WRKY* transcription factors. Furthermore, mycorrhizal fungal treatment increased root SA concentration but decreased root JA concentration under the condition of *P. parasitica* infection, indicating that mycorrhizal trifoliate orange plants preferred MAPK cascades and SA-mediated signal pathways to enhance the resistance to root rot.

The present work indicated that AMF inoculation dramatically increased root SA concentrations and the expression of root SA synthesis genes *PtPAL1* and *PtEPS1*, regardless

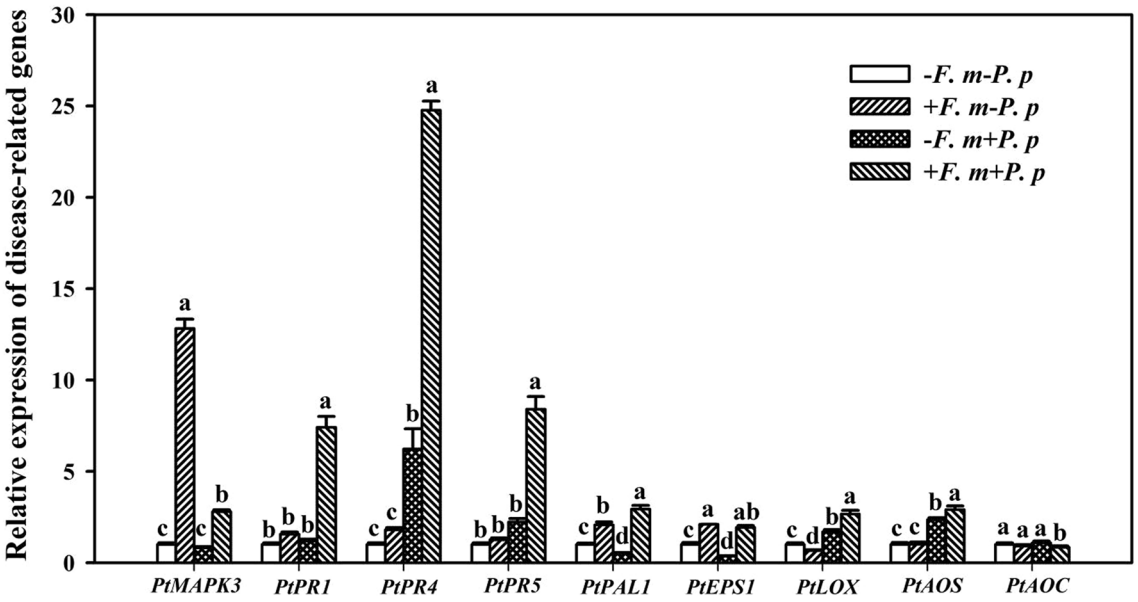

Fig. 2 Effects of *Funneliformis mosseae* inoculation and *Phytophthora parasitica* infection on the relative quantities of root defence-related genes in *Poncirus trifoliata*. The expression quantities of $+F.m-P.p$, $-F.m+P.p$, and $+F.m+P.p$ treatments were compared with $-F.m-P.p$ treatment. Data (means ± SD, $n=3$) followed by different letters above the bars indicate significant differences ($p<0.05$) between treatments. *F.m* inoculation with *Funneliformis mosseae*, and *P.p* infection by *Phytophthora parasitica*

of the infection with or without *P. parasitica*. As reported by Zhang et al. (2013), AMF inoculation stimulated the phenolic synthesis through regulating SA signaling pathways. Park et al. (2009) reported that the SA-dependent signaling activated the expression of PRs in plants. In our study, AMF-inoculated treatment up-regulated the root *PtPR1*, *PtPR4*, and *PtPR5* expression, especially in the plants with *P. parasitica* infection. Among PRs, PR1 has antifungal properties against several plant pathogenic fungi including *Phytophthora* spp. (Liu et al. 2019), and PR5 confers immunity from pathogen attacks (Monterio et al. 2003). Therefore, it was concluded that AMF induced expressions of *PRs* in host plants, which are of critical importance to resist root rot.

In addition to SA, JA also transfers the signal of pathogen attack to elicit plant defence responses (Sanders et al. 2000). Mehari et al. (2015) inoculated *Botrytis cinerea* on tomato plants and found that the plants were dependent on the JA response system to resist the pathogen. Besides, JA was involved in the resistance of *Brassica napus* to *Plasmodiophora brassicae* infection by *Heteroconium chaetospira* (Lahlali et al. 2014). While in the present work, compared with non-AM plants, AM plants without *P. parasitica* infection showed lower JA concentrations of roots, along with the down-regulation of *PtLOX* associated with JA synthesis. This is consistent with previous results conducted by Zhang et al. (2017), who found the inhibitive expression of *PtLOX* and the reduction of JA in trifoliate orange inoculated with three AMF species. In *P. parasitica*-infected plants, AMF treatment induced lower root JA concentrations but up-regulated the expression of root JA synthesis genes *PtLOX* and *PtAOS* (but not *PtAOC*). This suggests a distinct mechanism regarding the JA reduction after mycorrhization. SA and JA are antagonistic phytohormones that are controlled by transcription factors (Robert-Seilaniantz et al. 2011). The up-regulated expression of root *PtMPK3* in *P. parasitica*-infected plants caused by mycorrhization indicated an activated response in SA–JA systems. Therefore, it was speculated that under the condition of *P. parasitica* infection, SA accumulation induced by AMF reversely inhibited JA synthesis genes in the downstream, which further influenced JA biosynthesis. Further studies still have to be analyzed focusing on the relation between JA and SA.

NO as a plant signal molecule activates defensive gene expression (Nagai et al. 2020). In the present work, AM plants presented lower root NO concentrations compared with non-AM plants in spite of the infection with and without *P. parasitica*. This might be because SA appears to activate antioxidant systems which inhibit NO production (Farivar and Brecher 1996). It is reported that AMF increased CaM (a signal in regulation of physiological processes) content to reduce the oxidative damage in drought-stressed citrus plants (Huang et al. 2014). The present study showed a significantly higher root CaM level in AMF-treated plants versus non-AMF-treated plants, regardless of *P. parasitica* infection. Xie et al. (2019) also observed the increase of CaM concentrations in roots of *Xanthomonas amonopodis*-infected trifoliate orange after inoculation with *Paraglomus occultum*. It suggests that

mycorrhizal trifoliate orange is more likely dependent on CaM, but not NO, to transfer signals of the pathogen infection.

In this work, root chitinase and β-1,3-glucanase activities were induced by AMF inoculation irrespective of *P. parasitica* infection, indicating a better capacity to hydrolyze cell walls of *P. parasitica* in AM plants (Esquerré-Tugayé et al. 2000). Plant resistance to pathogenic fungi would be strengthened if the two enzymes work synergistically (Jongedijk et al. 1995; Hu et al. 2017). PAL takes part in the secondary metabolic process of phytoalexin, lignin, and phenolic compounds, and thus exerts the important role in resisting plant disease (Kim and Hwang 2014). In our study, AMF induced higher expression of root *PtPAL1*, accompanied with higher root PAL activities, no matter if infected with *P. parasitica* or not. Moreover, AMF induced significantly higher root TSP and lignin contents than non-mycorrhizal fungal treatment. Lignin increases the thickness of plant cell walls, constituting a mechanical barrier against pathogen invasion. Phenolic compounds restrain activities of pathogen polysaccharide enzymes, and accordingly reduce the destruction of host cell walls by pathogens further inhibiting pathogen infection (Du 2016). It was concluded that mycorrhizal trifoliate orange displayed higher *PtPAL1* expression and subsequent PAL activities, which may accelerate the generation of TSP and lignin to suppress *P. parasitica* infection in citrus.

Conclusion

Funneliformis mosseae inoculation activated the MAPKs cascades to amplify and transfer the signals of *P. parasitica* infection and cross-talk with SA-mediated signal pathways, to enhance plant disease resistance via increased expression of defence genes, higher levels of disease-related proteins, and higher concentrations of other compounds implicated in disease resistance. As a result, in field, greater AM status in citrus plants will benefit the enhancement in resistance of root rot through field management. More studies under field conditions need to be undertaken to analyze AMF functioning on tolerating citrus root rot.

Author contribution statement QSW and KK conceived the experiment. LT and YNZ conducted the experiment and analyzed the experimental data. LT wrote the first draft, and QSW revised the manuscript. All authors reviewed and approved the final draft.

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