Sains Malaysiana 47(8)(2018): 1693–1700
http://dx.doi.org/10.17576/jsm-2018-4708-08

Responses of Four Citrus Plants to *Phytophthora*-Induced Root Rot

(Tindak Balas Empat Tumbuhan Sitrus kepada Reput Akar Diinduksi dengan *Phytophthora*)

LI TIAN, QIANG-SHENG WU*, KAMIL KUČA & MOHAMMED MAHABUBUR RAHMAN

ABSTRACT

China is one of the largest citrus producers in Asia, where Phytophthora parasitica *infection has become the major threat in sustaining long term citrus production. The proposed study examined the effects of* P. parasitica *on* Citrus junos, C. limon, C. tangerina *and* Poncirus trifoliata *to evaluate the resisted rootstock to* Phytophthora *root rot.* P. parasitica *infection notably decreased plant growth, root morphology and activities of pathogenesis-related proteins (PRs) in* C. limon *and* C. tangerina. *Root β-1,3-glucanase, chitinase and phenylalanine ammonialyase activities significantly increased in* C. junos *and* P. trifoliata *after infection with* P. parasitica. P. parasitica *infection notably decreased root salicylic acid concentrations in* C. limon, C. tangerina *and* P. trifoliata, *while increasing it in* C. junos. *An opposite trend was observed in root jasmonic acid levels after infection with* P. parasitica, *relative to root salicylic acid. Root nitric oxide and calmodulin concentrations were significantly increased in* P. parasitica-*infected* C. junos, C. tangerina *and* P. trifoliata, *while* C. limon *exhibited a decrease. These results demonstrated that citrus species like* C. junos *and* P. trifoliata *displayed a much higher resistance to* Phytophthora-*induced root rot, and* C. limon *and* C. tangerina *showed a comparatively lower degree of resistance.*

Keywords: Citrus junos*; pathogenesis-related protein;* Phytophthora parasitica*;* Poncirus trifoliata

ABSTRAK

Negara China ialah salah sebuah negara pengeluar sitrus terbesar di Asia dengan jangkitan Phytophthora parasitica *telah menjadi ancaman utama dalam mengekalkan pengeluaran sitrus berjangka panjang. Kajian yang dicadangkan untuk mengkaji kesan* P. parasitica *pada* Citrus junos, C. limon, C. tangerina *dan* Poncirus trifoliata *bagi menilai akar umbi yang ditentang terhadap reput akar* Phytophthora. *Jangkitan* P. parasitica *terutamanya menurunkan pertumbuhan tumbuhan, morfologi akar dan aktiviti protein yang berkaitan patogenesis (PR) pada* C. limon *dan* C. tangerina. *Akar β-1,3-glukanase, kitinase and fenilalanina ammonia-liase meningkat dengan ketara pada* C. junos *dan* P. trifoliata *selepas jangkitan* P. parasitica. P. parasitica *terutamanya menurunkan kepekatan asid salisilik akar dalam* C. limon, C. tangerina *dan* P. trifoliata *manakala meningkatkannya dalam* C. junos. *Tren yang bertentangan telah diperhatikan pada peringkat asid jasmonik akar selepas jangkitan dengan* P. parasitica *berbanding dengan asid salisilat akar. Kepekatan nitrik oksida dan kalmodulin akar meningkat dengan ketara pada* P. parasitica *yang dijangkiti* C. junos, C. tangerina *dan* P. trifoliata *manakala* C. limon *menunjukkan suatu penurunan. Keputusan ini menunjukkan bahawa spesies sitrus seperti* C. junos *dan* P. trifoliata *menunjukkan rintangan yang lebih tinggi terhadap reput akar diinduksi dengan* Phytophthora *serta* C. limon *dan* C. tangerina *menunjukkan tahap rintangan yang agak rendah.*

Kata kunci: Citrus junos*;* Phytophthora parasitica*;* Poncirus trifoliata*; protein yang berkaitan patogenesis*

INTRODUCTION

Citrus is one of the important economic crops in the world, which is susceptible to a large number of diseases. *Phytophthora*-induced root rot is one of the most serious and destructive citrus diseases, considered to be the main obstacle towards stable high yield of citrus (Yan et al. 2017). In the early stage of root rot infection, the infection site presents irregularly wet and rot, resulting in xylem decay with pathogen extension (Yan et al. 2017).

The predominant pathogen of citrus root rot was identified as *Phytophthora* spp., primarily including *P. cactorum*, *P. capsici*, *P. citrophthora*, *P. palmvora* and *P. parasitica* (Zhou 1999), among which *P. parasitica* was reported as the main pathogen of citrus root rot in China (Liu et al. 1993; Zhou 1999). Infected trees usually lose vigor and may die prematurely (Queiroz et al. 2006). The soil-borne pathogen causes a multicyclic disease, living on both dead (as necrotrophic) and live (as biotrophic) plant organs and reproducing both sexually and asexually (Bonnet et al. 2007). The current practices for controlling *Phytophthora* diseases are largely based on improved cultivation practices in fields and application of synthetic fungicides (metalaxyl and fosetyl AI) (Gray et al. 2017; Latijnhouwers et al. 2003). However, long term application of chemical pesticides for the control of plant pathogens can trigger threatening levels of toxins in the environment and adverse effects on human health. Hence, it is particularly crucial to find the citrus rootstock against *Phytophthora*-induced root rot.

The present work was made to infect *P. parasitica* into four citrus genotype plants to identify a resistant citrus rootstock against *Phytophthora*-induced root rot by comparing plant growth performance, root morphology, activities of pathogenesis-related proteins (PRs) and signal substance concentrations.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The experiment was carried out in a completely randomized design with eight treatments: orange (*Citrus junos* Sieb. ex Tanaka) without *P. parasitica* infection (*C. j-P. p*), orange with *P. parasitica* infection (*C. j+P. p*), lemon [*Citrus limon* (L.) Burm. F.] without *P. parasitica* infection (*C. l-P. p*), lemon with *P. parasitica* infection (*C. l+P. p*), tangerine (*Citrus tangerina* Hort. ex Tanaka) without *P. parasitica* infection (*C. t-P. p*), tangerine with *P. parasitica* infection (*C. t+P. p*), trifoliate orange [*Poncirus trifoliata* (L.) Raf.] without *P. parasitica* infection (*P. t-P. p*) and trifoliate orange with *P. parasitica* infection (*P. t+P. p*). The four citrus plants are major rootstocks used for commercial cultivation of Satsuma mandarin in China. Each treatment was replicated five times, with a total of 40 pots, and one citrus seedling per pot.

PLANT CULTURE

Seeds of *C. junos*, *C. limon*, *C. tangerina* and *P. trifoliata* were sterilized with 70% alcohol solution for 10 min and then germinated on autoclaved (0.11 MPa, 121ºC, 2 h) sands under the conditions of 28/20ºC day/night temperature and 80% relative air humidity. Subsequently, one 3-leaf-old seedling with uniform size was transplanted into the plastic pot (13.5 cm upper diameter × 9.5 cm height × 11 cm bottom diameter), supplied with 1.5 kg of autoclaved (0.11 MPa, 121ºC, 2 h) substrates (soil : sand = 5 : 1, v/v). All these pots were placed in a plastic greenhouse of Yangtze University campus. The growing conditions of greenhouse were characterized by photon flux density of 721-967 μmol/m^2/s with 25/19ºC average day/night temperature and 75-95% relative air humidity.

PATHOGEN INFECTION

P. parasitica was freely provided by the Citrus Research Institute, Chinese Academy of Agricultural Sciences. *P. parasitica* was grown in PDA medium at 28ºC for 7 days for the pathogen infection. After 8 weeks of transplanting, the sclertium of *P. parasitica* (5 × 5 mm) was applied on the root neck of four citrus plants according to the protocol proposed by Zhou (1999).

OBSERVATIONS AND ANALYTICAL METHODS

A week later of the pathogen infection, all the citrus plants were harvested and divided into shoots and roots, whose fresh weight was measured. Height, stem diameter and leaf number were measured before plant was harvested. The root system of each plant was scanned by the Epson Perfection V700 Photo Dual Lens System (J221A, Indonesia). Root morphological traits, including length, diameter, projected area, surface area and volume were analyzed with the scanned photo by a WinRHIZO professional software in 2007b (Regent Instruments Inc., Quebec, Canada).

Root β-1,3-glucanase activity was determined by the method of Hu et al. (2017). An enzyme activity unit was defined as enzyme amounts required for catalyzing laminarin and releasing 1 μg glucose by 1 g of fresh tissue per minute in certain conditions (37ºC, pH5.0).

Root chitinase activity was measured according to the method of Hu et al. (2017). An enzyme activity unit was defined as enzyme amounts required for catalyzing colloidal chitin and releasing 1 μmol N-acetylglucosamine (NAG) by 1 g of fresh tissue per minute in certain conditions (37ºC, pH5.0).

Root phenylalanine ammonia-lyase (PAL) activity was assayed using the method of Kofalvi and Nassuth (1995), where an enzyme activity unit was defined as enzyme amounts required for optical density (OD) change of 0.01 unit per hour at 290 nm.

Root salicylic acid (SA), jasmonic acid (JA), nitric oxide (NO) and calmodulin (CaM) concentrations were assayed by double antibody sandwich-elisa kits in ELISA as per the user handbook (Shanghai Enzyme Linked Biotechnology Co., Ltd, China).

STATISTICAL ANALYSIS

Data (means ± SD, $n = 8$) were analyzed with ANOVA (SAS, version 8.1) and the significant differences among these treatments were compared with the Fisher's Protected Least Significant Difference (LSD) at $p < 0.05$.

RESULTS

PLANT GROWTH PERFORMANCE

Treatment with *P. parasitica* significantly reduced the growth performance of different citrus plants, as compared with non-infected treatment (Table 1). *P. parasitica* infection dramatically decreased plant height, stem diameter, leaf number, shoot biomass and root biomass by 4%, 12%, 11%, 9% and 14% in *C. tangerina* and by 9%, 8%, 13%, 20% and 21% in *C. limon*, respectively. In *C. junos* and *P. trifoliata*, plant height, stem diameter, shoot biomass and root biomass significantly reduced by 3%, 3%, 9% and 15% under *P. parasitica* condition and by 4%, 2%, 9% and 14% under *P. parasitica* condition, respectively. Leaf number was not significantly affected by the pathogen infection.

TABLE 1. Effects of *Phytophthora parasitica* infection on plant growth of *Citrus junos*, *C. limon*, *C. tangerina* and *Poncirus trifoliata* seedlings

Citrus plants	*Phytophthora parasitica* treatments	Plant height (cm)	Stem diameter (mm)	Leaf number per plant	Shoot biomass (g DW/plant)	Root biomass (g DW/plant)
Citrus junos	*-P. p*	10.71±0.49c	1.94±0.11cd	11±0.69b	1.13±0.01a	0.33±0.03c
	+P. p	9.36±0.69d	1.88±0.11d	11±0.69b	0.87±0.07cd	0.28±0.03d
C. limon	*-P. p*	9.36±0.56d	1.71±0.13e	8±0.82d	0.54±0.03e	0.34±0.03c
	+P. p	8.43±0.61e	1.58±0.07f	7±0.69d	0.53±0.05e	0.27±0.02d
C. tangerina	*-P. p*	9.57±0.35d	2.21±0.12a	9±0.69c	0.94±0.08bc	0.63±0.04a
	+P. p	9.14±0.24ed	1.94±0.15bcd	8±0.53cd	0.85±0.04d	0.54±0.03b
Poncirus trifoliata	*-P. p*	18.54±0.99a	2.07±0.16b	15±1.27a	0.96±0.05b	0.66±0.03a
	+P. p	17.75±1.01b	2.03±0.16bc	15±1.15a	0.87±0.08cd	0.57±0.04b

Data (means ± SD, n = 8) followed by different letters in a row indicate significant differences (p< 0.05) between treatments. Abbreviation: *-P. p*, non-infection by *Phytophthora parasitica*; *+P. p*, infection by *Phytophthora parasitica*

ROOT MORPHOLOGY

Compared with non-infected treatment, infection with *P. parasitica* prominently showed a reduction in root total length, projected area and surface area by 4%, 12% and 4% in *C. tangerina*, 34%, 24% and 24% in *C. limon* and 8%, 9%, and 10% in *P. trifoliata*, respectively (Table 2). *C. junos* had no significant response to infection with *P. parasitica* with regard to root total length, projected area and volume. Furthermore, compared with non-infected treatment, *P. parasitica* infection dramatically decreased the number of 1st- and 2nd- order lateral roots (LRs) by 6% and 22% in *C. tangerina*, by 6% and 63% in *C. limon* and by 11% and 36% in *P. trifoliata* (Table 2). There were no changes in the 3rd-order LR in *C. tangerina*, *C. limon* and *C. junos*, except for *P. trifoliata*, relative to non-infected seedlings.

ACTIVITIES OF ROOT PATHOGENESIS-RELATED PROTEINS

The pathogen infection distinctively increased root β-1,3-glucanase activities as compared with the non-infected treatment, registering as much as 19% higher activity in *C. junos* and 17% higher in *P. trifoliata*, respectively (Figure 1(a)). However, in comparison with non-infected treatment, root β-1,3-glucanase activities decreased evidently by 16% and 13% under the condition of *P. parasitica* in *C. limon* and *C. tangerina*, respectively.

In comparison with non-infected treatments, root chitinase activities decreased prominently by 13% and 22% in *C. tangerina* and *C. limon* when infected with *P. parasitica* (Figure 1(b)). Besides, *P. parasitica* infection prominently increased root chitinase activities by 22% and 5% in *C. junos* and *P. trifoliata,* respectively, as compared with non-infected seedlings.

Compared with non-infected treatment, *P. parasitica* infection significantly reduced root PAL activities by 45% in *C. tangerina* and 9% in *C. limon*, respectively (Figure 1(c)). Besides, the pathogen infection dramatically promoted root PAL activities by 67% in *C. junos* and by 56% in *P. trifoliata*, respectively, as compared with non-infected treatment.

CONCENTRATIONS OF ROOT SIGNAL SUBSTANCES

P. parasitica infection dramatically decreased root SA concentrations by 30% in *C. tangerina* and 26% in *C. limon*, respectively, in contrast with non-infected seedlings (Figure 2(a)). There were no significant differences in root SA contents between infected and non-infected *P. trifoliata*. Moreover, root SA concentrations prominently increased by 10% in infected *C. junos* in comparison with non-infected seedlings.

Compared with non-infected treatment, *P. parasitica* infection dramatically elevated root JA concentrations: 32% higher in *C. tangerina*, 35% in *C. limon* and 34% in *P. trifoliata* (Figure 2(b)).

Root NO contents were decreased by 5% in infected *C. limon*, as compared with non-infected *C. limon* (Figure 2(c)). Additionally, *P. parasitica* infection evidently improved root NO concentrations by 5% in *C. tangerina*, 31% in *C. junos* and 21% in *P. trifoliata*, respectively, as compared with non-infected seedlings.

In comparison with non-infected treatment, *P. parasitica* infection notably increased root CaM concentrations: 55% higher in *C. tangerina*, 41% in *C. junos* and 12% in *P. trifoliata*, respectively (Figure 2(d)). Root CaM contents in *P. parasitica*-infected *C. limon* were dramatically reduced by 24% in relation to non-infected plants.

DISCUSSION

In this study, plant height, stem diameter, shoot biomass and root biomass of four citrus plants infected with *P. parasitica* were significantly lower than those non-infected with *P. parasitica*. This is in accordance with earlier results conducted by Davis and Menge (1980), who reported that sole *P. parasitica* infection dramatically decreased plant growth performance than non-infected sweet orange. This manifested that *P. parasitica* infection had the negative effect on plant growth traits of citrus plants. Moreover, taking the relative reduction of plant growth indexes into

TABLE 2. Effects of *Phytophthora parasitica* infection on root morphological traits of *Citrus junos*, *C. limon*, *C. tangerina* and *Poncirus trifoliata* seedlings

Citrus plants	*Phytophthora parasitica* treatments	Total length (mm)	Average diameter (mm)	Projected area (cm^2)	Surface area (cm^2)	Volume (cm^3)	Number of lateral roots		
							1st-order	2nd-order	3rd-order
Citrus junos	-*P. p*	82.07±6.43d	0.58±0.02a	4.69±0.07e	14.70±0.73d	0.21±0.01c	24.50±2.08d	13.00±1.15f	1.00±0.82c
	+*P. p*	81.34±7.10d	0.57±0.02a	4.28±0.17e	13.45±0.65e	0.20±0.01c	22.25±1.71d	18.00±0.82de	0.00±0.00d
C. limon	-*P. p*	84.47±2.45d	0.56±0.01b	4.35±0.15e	13.66±0.21de	0.18±0.01d	34.00±2.08ab	17.50±0.58e	1.25±0.50c
	+*P. p*	56.17±5.37e	0.53±0.03b	3.32±0.21f	10.43±0.42f	0.16±0.01d	32.00±1.63bc	6.50±0.58g	0.00±0.00d
C. tangerina	-*P. p*	135.13±3.08c	0.58±0.03a	8.63±0.17c	24.74±0.22c	0.36±0.02b	31.75±2.06bc	28.00±2.16c	1.00±0.82c
	+*P. p*	130.23±7.11c	0.58±0.03a	7.58±0.01d	23.81±0.23c	0.38±0.03b	30.00±1.83c	21.75±1.26d	0.00±0.00d
Poncirus trifoliata	-*P. p*	264.89±9.32a	0.43±0.02c	11.45±0.75a	35.96±1.92a	0.39±0.01a	36.88±3.27a	85.13±5.46a	9.88±0.64a
	+*P. p*	243.20±11.92b	0.42±0.02c	10.4±0.45b	32.48±1.58b	0.38±0.02a	33.00±2.56bc	54.75±4.27b	2.75±0.89b

Data (means ± SD, $n = 8$) followed by different letters in a row indicate significant differences ($p<0.05$) between treatments. Abbreviation: -*P. p*, non-infection by *Phytophthora parasitica*; +*P. p*, infection by *Phytophthora parasitica*

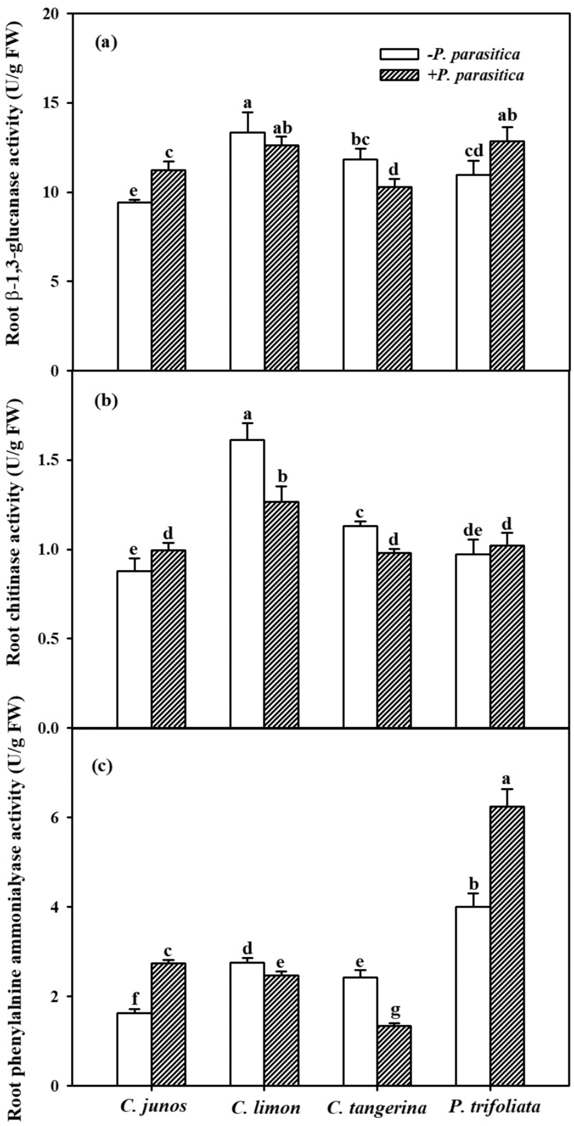

FIGURE 1. Effects of *Phytophthora parasitica* infection on root β-1,3-glucanase (a), chitinase (b) and phenylalnine ammonialyase (c) activities in *Citrus junos*, *C. limon*, *C. tangerine* and *Poncirus trifoliata*. Data (means ± SD, *n* = 8) are significantly different (p<0.05) followed by different letters above the bars. Abbreviation: -*P. parasitica*, non-infection by *Phytophthora parasitica*; +*P. parasitica*, infection by *Phytophthora parasitica*

consideration, *C. junos* and *P. trifoliata* performed better than *C. tangerina* and *C. limon*, suggesting that *C. junos* and *P. trifoliata* had higher resistance to *Phytophthora* root rot than the other two citrus plants.

In the present study, *P. parasitica* infection notably reduced root morphological traits of citrus plants than those of non-inoculated plants, apart from *C. junos*, whose root morphological traits were less affected by *P. parasitica*. This suggested that *C. junos* presented high tolerance to root rot by *P. parasitica*. Besides, the number of LRs in *P. parasitica*-infected plants was evidently lower than in non-infected plants, except for the 2nd-order LRs in *C. junos*. As reported by Vallad (2004), plant protected themselves from pathogenic damage by activating systemic acquired resistance (SAR). It was suggested that *C. junos* exhibited stronger resistance to *Phytophthora* root rot from the relatively higher LR numbers induced by pathogen infection. In addition, *P. trifoliata* exhibited the highest root morphological traits than the other citrus plants, regardless of infection with *P. parasitica* or not. It appeared to show higher resistance to *Phytophthora* root rot. Considering the relative reduction of root morphological traits under *P. parasitica*, the severity of disease in *P. trifoliata* was greater than *C. tangerina,* implying an inferior resisted capacity of *P. trifoliata* relative to *C. junos*. In short, in terms of root morphology, the resisted capacity to *Phytophthora* root rot among these citrus plants was ranked as *C. junos* > *P. trifoliata* > *C. tangerina* > *C. limon* in the decreasing order.

In general, the changes in β-1,3-glucanase and chitinase activities are obvious synchronicity (Hu et al. 2017). In this work, the variation tendency of both root

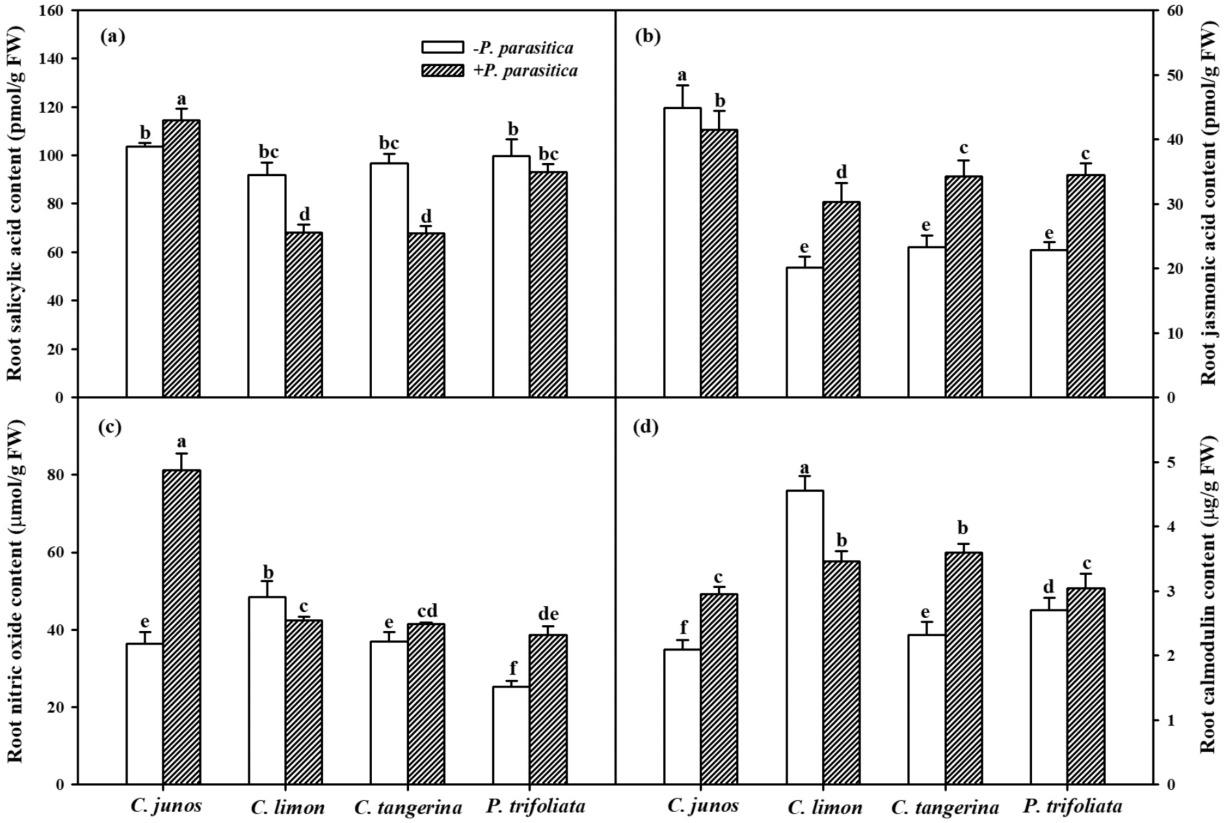

FIGURE 2. Effects of *Phytophthora parasitica* infection on root salicylic acid (a), jasmonic acid (b), nitric oxide (c) and calmodulin (d) concentrations in *Citrus junos*, *C. limon*, *C. tangerine* and *Poncirus trifoliata*. Data (means ± SD, $n = 8$) are significantly different ($p<0.05$) followed by different letters above the bars. Abbreviation: -*P. parasitica*, non-infection by *Phytophthora parasitica*; +*P. parasitica*, infection by *Phytophthora parasitica*

β-1,3-glucanase and chitinase activities were similar as a whole in different citrus plants infected by *P. parasitica*. In fact, β-1,3-glucanase and chitinase could hydrolyze the cell wall of pathogenic fungi (Esquerré-Tugayé et al. 2000). In addition, the hydrolytic process of cell wall for pathogen fungi is accompanied with oligose release, which acts as a motivational factor of multiple disease resistance response (Klarzynski et al. 2000). In the present work, *P. parasitica* infection considerably increased root β-1,3-glucanase and chitinase activities in *C. junos* and *P. trifoliata*, while decreasing that in *C. tangerina* and *C. limon*. Earlier studies confirmed that the expression levels of *GmBG1* and *MdChi* were higher in disease-resistant varieties than that in susceptible varieties (Li et al. 2003; Zhang et al. 2017). Hence, it could be speculated that *C. junos* and *P. trifoliata* presented higher resistance to *Phytophthora* root rot than *C. tangerina* and *C. limon* through hydrolyzing the cell wall of *P. parasitica*, especially *C. junos*.

PAL acts as the key enzyme and limiting enzyme in the secondary metabolism pathway of phenypropanoids, exerting the crucial effect on plant disease resistance (Kim & Hwang 2014). The present study indicated that root PAL activities were increased prominently when these citrus plants were infected by the pathogen. In fact, earlier studies showed the larger and faster increase in PAL activity in resistant cultivars than in susceptible cultivars (Dixon et al. 2002; Mozzetti et al. 1995; Shiraishi et al. 1995). In this work, *P. parasitica* infection considerably reduced root PAL activities in *C. tangerina* and *C. limon* compared with non-infected seedlings, suggesting that *C. tangerina* and *C. limon* showed lower resistance to *P. parasitica*-induced root rot. In contrast, root PAL activities were highly promoted by *P. parasitica* infection in *C. junos* and *P. trifoliata*, especially *P. trifoliata*, impling that *P. trifoliata* and *C. junos* performed better resistance than *C. tangerina* and *C. limon*.

SA and JA can transfer the signals of wounding, pathogens and herbivores attack to trigger the defense responses in plants (Sanders et al. 2000; Wasternack & Parthier 1997). The SA signals exhibit a more durable and intense response to pathogen infection (Song et al. 2010). In this work, root SA concentrations increased dramatically in *C. junos* by the pathogen infection, while decreased in the other citrus plants, indicating an oppositely changed trend between root SA concentrations and root JA concentrations by *P. parasitica* infection in these citrus plants. It is known that SA inhibits JA biosynthesis and defense responses (Glazebrook 2001; Robert-Seilaniantz et al. 2011) and the inhibition is always modulated by transcription factors such as *WRKY70*, *WRKY62*, *MPK4*, *MYC2* and *NPR1* (Bari & Jones 2009). The present results suggested that *C. junos* responded to pathogen attack primarily by means of increasing SA concentration, while *C. limon*, *C. tangerina* and *P. trifoliata* preferred to accumulate root JA contents to defend pathogen infection. As a result, citrus plants possess differently responsive patterns in SA and JA to *P. parasitica* infection.

NO generally takes part in the post-translational modification of proteins involving stress, redox and signaling (Lindermayr et al. 2005). The complex of calcium (Ca^{2+}) and CaM can regulate physiological processes in plants (Kim et al. 2009). In our work, apart from *C. limon*, whose root NO and CaM contents reduced dramatically after infection by *P. parasitica*. *P. parasitica* treatment strongly increased root NO and CaM concentrations in *C. junos*, *C. tangerina* and *P. trifoliata*. With regard to the relative growth rate, however, *C. junos* was notably higher than *C. tangerine* and *P. trifoliata*, which meant that *C. junos* presented stronger response to the pathogen infection through NO and CaM accumulation in roots.

Conclusion

Infection with *P. parasitica* notably reduced the plant growth and root morphology of four citrus plants, whilst the magnitude of reduction was higher in *C. limon* and *C. tangerina* than in *C. junos* and *P. trifoliata*, especially *C. limon*. Besides, activities of PRs were significantly reduced in *C. limon* and *C. tangerina* after *P. parasitica* infection and increased in *C. junos* and *P. trifoliata,* especially *C. junos*. The pathogen infection also stimulated SA, NO and CaM accumulation in *C. junos* and JA, NO, CaM accumulation in *P. trifoliata*. These observations provide the strong evidence that *C. junos* exhibited the highest resistance to *Phytophthora*-induced root rot, followed by *P. trifoliata*, *C. tangerina* and *C. limon* in decreasing order of the resistance.

ACKNOWLEDGEMENTS

This study was supported by the Plan in Scientific and Technological Innovation Team of Outstanding Young Scientist, Hubei Provincial Department of Education (T201604). It was also supported by the Long-Term Development Plan of UHK.

REFERENCES

Bari, R. & Jones, J.D. 2009. Role of plant hormones in plant defence responses. *Plant Molecular Biology* 69(4): 473-488.

Bonnet, J., Danan, S., Boudet, C., Barchi, L., Sage-Palloix, A. & Caromel, B. 2007. Are the polygenic architectures of resistance of *Phytophthora capsici* and *P. parasitica* independent in pepper? *Theoreticao & Appied Genetic* 115(2): 253-264.

Davis, R.M. & Menge, J.A. 1980. Influence of *Glomus fasciculatus* and soil phosphorus on *Phytophthora* root rot of citrus. *Phytopathology* 70(5): 447-452.

Dixon, R.A., Achnine, L., Kota, P., Liu, C.J., Reddy, M.S. & Wang, L. 2002. The phenylpropanoid pathway and plant defence-a genomics perspective. *Molecular Plant Pathology* 3(5): 371-390.

Esquerré-Tugayé, M.T., Boudart, G. & Dumas, B. 2000. Cell wall degrading enzymes, inhibitory proteins, and oligosaccharides participate in the molecular dialogue between plants and pathogens. *Plant Physiology and Biochemistry* 38(1): 157-163.

Glazebrook, J. 2001. Genes controlling expression of defense responses in Arabidopsis-2001 status. *Current Opinion in Plant Biology* 4(4): 301-308.

Gray, M.A., Hao, W., Forster, H. & Adaskaveg, J.E. 2017. Effect of new Oomycete-specific fungicides on tree health, fruit yields and *Phytophthora* root rot of citrus. *Phytopathology* 107: 186.

Hu, N., Tu, X.R., Li, K.T., Ding, H., Li, H., Zhang, H.W., Tu, G.Q. & Huang, L. 2017. Changes in protein content and chitinase and β-1,3-glucanase activities of rice with blast resistance induced by Ag-antibiotic 702. *Plant Diseases and Pests* 8(4): 33-36.

Kim, D.S. & Hwang, B.K. 2014. An important role of the pepper phenylalanine ammonia-lyase gene (*PAL1*) in salicylic acid-dependent signalling of the defence response to microbial pathogens. *Journal of Experimental Botany* 65(9): 2295-2306.

Kim, M.C., Chung, W.S., Yun, D.J. & Cho, M.J. 2009. Calcium and calmodulin-mediated regulation of gene expression in plants. *Molecular Plant* 2(1): 13-21.

Klarzynski, O., Plesse, B. & Joubert, J.M. 2000. Linear β-1,3-glucans are elicitors of defense responses in tobacco. *Plant Physiology* 124: 1027-1037.

Latijnhouwers, M., De Wit, P.J. & Govers, F. 2003. Oomycetes and fungi: Similar weaponry to attack plants. *Trends in Microbiology* 11(10): 462-469.

Li, Y.Z., Zheng, X.H., Tang, H.L., Zhu, J.W. & Yang, J.M. 2003. Increase of β-1,3-glucanase and chitinase activities in cotton callus cells treated by salicylic acid and toxin of *Verticillium dahliae*. *Acta Botanica Sinica* 45(7): 802-808.

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

Liu, J.J. & Yin, G.Y. 1993. Study on the root rot and yellow leaf disease of citrus in Jiangsu and Hubei provinces. *Journal of Nanjing Agricultural University* 16(1): 38-44 (in Chinese with English abstract).

Mozzetti, C., Ferraris, L., Tamietti, G. & Matta, A. 1995. Variation in enzyme activities in leaves and cell suspensions as markers of incompatibility in different *Phytophthora*-pepper interactions. *Physiological and Molecular Plant Pathology* 46(2): 95-107.

Queiroz, B.P.V. & Melo, I.S. 2006. Antagonism of *Serratia marcescens* towards *Phytophthora parasitica* and its effects in promoting the growth of citrus. *Brazilian Journal of Microbiology* 37(4): 448-450.

Robert-Seilaniantz, A., Grant, M. & Jones, J.D. 2011. Hormone crosstalk in plant disease and defense: More than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology* 49: 317-343.

Sanders, P.M., Lee, P.Y., Biesgen, C., Boone, J.D., Beals, T.P., Weiler, E.W. & Goldberg, R.B. 2000. The Arabidopsis *DELAYED DEHISCENCE1* gene encodes an enzyme in the jasmonic acid synthesis pathway. *The Plant Cell* 12(7): 1041-1061.

Shiraishi, T., Yamada, T., Nicholson, R.L. & Kunoh, H. 1995. Phenylalanine ammonia-lyase in barley: Activity enhancement in response to *Erysiphe graminis* f. sp. hordei (race 1) a pathogen, and *Erysiphe pisi*, a nonpathogen. *Physiological and Molecular Plant Pathology* 46(2): 153-162.

Song, Y.Y., Zeng, R.S., Xu, J.F., Li, J., Shen, X. & Yihdego, W.G. 2010. Interplant communication of tomato plants through underground common mycorrhizal networks. *PloS ONE* 5(10): e13324.

Vallad, G.E. & Goodman, R.M. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science* 44(6): 1920-1934.

Wasternack, C. & Parthier, B. 1997. Jasmonate-signalled plant gene expression. *Trends in Plant Science* 2(8): 302-307.

Yan, H.X., Zhong, Y., Jiang, B., Zhou, B.R., Wu, B. & Zhong, GY. 2017. Guanggan (*Citrus reticulata*) shows strong resistance to *Phytophthora nicotianae*. *Scientia Horticulturae* 228: 141-149.

Zhang, L., Wei, L., Tang, X.F., Wang, W.W., Yu, Z.Y. & Liu, L.J. 2017. Bioinformatics analysis of soybean β-1,3-glucanase (*GmBG1*) and its homologous proteins. *Genomics and Applied Biology* 36(3): 1035-1042.

Zhou, C.H. 1999. Resistance identification of citrus somatic cell hybrid to *Phytophthora parasitica* and study of *Phytophthora parasitica* toxin. Doctoral dissertation, Wuhan: Huazhong Agricultural University (Unpublished).

Li Tian & Qiang-Sheng Wu*
College of Horticulture and Gardening
Yangtze University
Jingzhou, Hubei 434025
China

Qiang-Sheng Wu* & Kamil Kuča
Department of Chemistry, Faculty of Science
University of Hradec Kralove
Hradec Kralove 50003
Czech Republic

Mohammed Mahabubur Rahman
Brix' N Berries
Leduc, Alberta
Canada

*Corresponding author; email: wuqiangsh@163.com

Received: 2 March 2018
Accepted: 4 April 2018