

Effect of Exogenous Phenolic Substances on Susceptibility of Octoploid Strawberry to Crown Rot

C. Luo¹, Y. Hu¹, and B. Shu^{1*}

ABSTRACT

Crown rot caused by *Colletotrichum siamense* is a serious disease of strawberry in the Yangtze River region, China. The metabolites involved in phenylpropanoid biosynthesis increase susceptibility of crown rot in octoploid strawberry. Exogenous coumaric acid, caffeic acid, and ferulic acid involved in phenylpropanoid biosynthesis were used to explore whether the increased susceptibility was associated with reactive oxygen species, antioxidant substance and key genes of phenylpropanoid metabolism. According to the results, H₂O₂, O₂⁻, and MDA contents showed different responses to *C. siamense* infection in root, petiole and leaf. The H₂O₂, O₂⁻ and MDA were increased by *C. siamense* in petiole. Exogenous coumaric acid, caffeic acid and ferulic acid promoted the H₂O₂, O₂⁻ and MDA as positive control. The POD activity was significantly induced in response to *C. siamense* infection in petiole. The coumaric acid treatment inhibited the POD activity but increased total phenolics. The ferulic acid only promoted POD activity in petiole. In addition, the expressions of transcripts involved in phenylpropanoid biosynthesis were regulated by exogenous coumaric acid, caffeic acid, and ferulic acid. Together, our results demonstrated that exogenous coumaric acid, caffeic acid, and ferulic acid increased susceptibility of octoploid strawberry to crown rot by regulated ROS, antioxidant substance, and transcripts expression of phenylpropanoid biosynthesis.

Keywords: Antioxidant substance, 'Benihoppe' strawberry, Gene expression, Phenylpropanoid, Reactive oxygen species.

INTRODUCTION

Strawberry (*Fragaria×ananassa*) is a popular berry worldwide, and crown rot threatens its production in nearly all production areas. Strawberry crown rot, caused by *Colletotrichum siamense*, is a serious disease of strawberry in Hubei Province, China (Luo *et al.*, 2021). Knowledge on the physiological process of strawberry responding to *C. siamense* is helpful for controlling the serious disease.

Reactive Oxygen Species (ROS) have been confirmed to act directly on pathogens and function as defensive gene signals and/or function molecules in plant defense responses (Oliveira *et al.*, 2016). Production

of O₂⁻ and H₂O₂ are well known to be involved in plant defense (Díaz-Vivancos *et al.*, 2010; Camejo *et al.*, 2011). One early defense reaction of plant to pathogen attack is the so-called "oxidative burst", which generates a localized and transient production of ROS. In addition to oxidative burst, H₂O₂ and O₂⁻ can accumulate during pathogen infection, and it is related to the levels of disease resistance. Accumulation of ROS has been found to control and inhibit the pathogen growth. ROS have been proposed to act as antimicrobial molecules, cross-linkers of the plant cell wall to block pathogen entry (Suzuki *et al.*, 2011; Nathan and Cunningham-Bussel, 2013; Gilroy *et al.*, 2014). However, excessive ROS will

¹ College of Horticulture and Gardening, Yangtze University, 434025 Jingzhou, P. R. China.

*Corresponding author; e-mail: bshbest@163.com



damage cells and destroy the integrity of the cell membrane (Ray *et al.*, 2012; Nath *et al.*, 2017). Plants may eliminate excess ROS through the antioxidant enzymes including Superoxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POD), and non-antioxidant enzymes like phenolics in phenylpropanoid pathway and soluble protein, which is critical for balancing ROS metabolism and plant resistance (de Freitas-Silva *et al.*, 2017; Zhu *et al.*, 2019).

Early and rapid accumulation of phenolic compounds at infection sites is involved with disease resistance in plant-pathogen interactions (Matern and Kneusel, 1988). The phenylpropanoid pathway plays an important role in plant disease resistance (Yong-Hong *et al.*, 2012). The regulation of phenylpropanoid metabolism promotes the accumulation of phenolic substances (coumaric acid, caffeic acid and ferulic acid) and affects the resistance of plant against pathogens (Deng *et al.*, 2015; Li *et al.*, 2017). Most studies showed several free phenols with antifungal activity against pathogen such as caffeic acid, which activated phenylpropanoid pathway as peroxidase and also increased the content of total phenols and inhibited gray mold effectively (Zhang *et al.*, 2020). Given that the molecules involved in phenylpropanoid pathway have diverse roles in plant defense, one might expect phenylpropanoid pathway inhibition to impair plant immunity. In contrast, the phenylpropanoid pathway inhibitor piperonylic acid induces broad-spectrum disease resistance in plants (Desmedt *et al.*, 2021). Our previous study observed metabolites involved in phenylpropanoid biosynthesis increased susceptibility of *C. siamense* causing crown rot in octoploid strawberry. It was not clear whether the susceptibility increment was related with ROS and phenylpropanoid pathway regulation or not.

In this study, we aimed to use exogenous coumaric acid, caffeic acid, and ferulic acid to explore the differences in reactive oxygen species and key genes of phenylpropanoid metabolism, thereby providing a

physiological basis for further studies on the resistance mechanism of phenolic substances to crown rot of strawberry.

MATERIALS AND METHODS

Five treatments (coumaric acid, caffeic acid, ferulic acid, negative control, and positive control) were used for ensuring effects of phenolic substances for strawberry crown rot caused by *C. siamense*. Each treatment contained seedlings of six pots (three seedlings in each pot) and the seedlings of each pot were taken as one biological replicate. The positive control seedlings and those seedlings that would be sprayed with coumaric acid, caffeic acid, ferulic acid were inoculated with *C. siamense* SCR-7, and the negative control seedlings were inoculated with nontoxic medium (Luo *et al.*, 2021). Twenty-four hour after inoculation, coumaric acid (250 mg L⁻¹), caffeic acid (75 mg L⁻¹) and ferulic acid (750 mg L⁻¹) was sprayed to each treatment every two days, respectively, while negative control and positive control were sprayed with sterile water. The root, petiole, and leaf of each biological replicate were sampled, on 6 days after inoculation.

The O₂⁻ concentration in crown was assayed using the protocol outlined by He *et al.* (2020). Fresh tissue (0.1 g) was homogenized in 5 mL of 0.1 mol L⁻¹ phosphate buffer (pH 7.8) and centrifuged at 4,000×g for 10 minutes at 4°C. The 0.5 mL of supernatant was mixed with 0.5 mL of 50 mmol L⁻¹ phosphate buffer and 0.1 mL of 10 mmol L⁻¹ hydroxylamine chloride for 1 hour at 25°C. Subsequently, 1 mL of 17 mmol L⁻¹ sulfanilamide and 1 mL of 7 mmol L⁻¹ α-naphthylamine were added to the mixture at 25°C for 20 minutes, and the absorbance was recorded at 530 nm.

The H₂O₂ concentrations in the crowns were determined using the method described by Velikova *et al.* (2000). Fresh samples (0.1 g) were homogenized with 5 mL of 0.1% (w/v) trichloroacetic acid and centrifuged at 12,000×g for 15 minutes. A 4 mL mixture was comprised of 1 mL of 10 mmol L⁻¹ potassium phosphate buffer (pH 7.0), 2 mL of 1 mol L⁻¹

KI, and 1 mL of supernatant, and the absorbance of the mixture was recorded at 390 nm.

Malonaldehyde (MDA), a by-product of oxidative damage to cells, was measured following the method described by Sudhakar *et al.* (2001). Fresh crowns (0.1 g) were homogenized with 5 mL of 0.1% (w/v) trichloroacetic acid and centrifuged at 3,000×g for 10 minutes. The mixture, including 2 mL of supernatant and 2 mL of 0.67% thiobarbituric acid, was incubated at 100°C for 30 minutes and then centrifuged at 3,000×g for 10 minutes. The absorbance was recorded at 450, 532, and 600 nm, respectively.

A fresh sample (0.5 g) was homogenized in 7 mL of 0.1M phosphate buffer, pH 7.8. Insoluble material was removed by centrifugation at 4,200×g for 10 min, with the resulting supernatant used for the assays of SOD, CAT and soluble protein. SOD activity was measured using the method described by Wu *et al.* (2006), and expressed in Unit g⁻¹ FW. One SOD unit is defined as the amount of enzyme that inhibits 50% nitro blue tetrazolium by light. CAT activity was measured using the method of He *et al.* (2019), one Unit (U) of CAT activity was defined as a 0.01 decrease in the absorbance at 240 nm in 1 minute. Soluble protein was measured as Bradford method using bovine serum albumin as the standard (Wu *et al.*, 2006).

The activity of POD was measured as described by Wu *et al.* (2018). Simply, 0.3 g sample was grounded into homogenates with 8 mL of 0.1 mmol L⁻¹ phosphate buffer (pH 5.5) and centrifuged for 10 min at 3,000×g. The mixture of 2.9 mL of phosphate buffer (pH 5.5), 1.0 mL of H₂O₂, 1.0 mL of 0.05 mol L⁻¹ guaiacol, and 0.1 mL of supernatant was orderly placed into a 10 mL centrifugal tube, which was immediately incubated at 34°C for 3 minutes. The absorbance of the mixture at 470 nm was recorded. A change in the absorbance of 0.01 in 1 min was defined as one enzyme unit.

The total phenolic was determined by the Folin-Ciocalteu method with a little modification (Li *et al.*, 2012). Firstly, 0.5 mL

extract methanolic solution (0.4 mg mL⁻¹) was mixed with 0.5 mL 0.25 mol L⁻¹ Folin-Ciocalteu reagent. After incubation for 3 minutes, 1 mL of Na₂CO₃ solution (15%, w/v) was added. After standing at the room temperature for 30 minutes, the mixture was centrifuged at 3,500×g for 3 minutes. The absorbance of the supernatant was measured at 760 nm (Unico 2100, Shanghai, China). The determinations were performed in triplicate, and the calculations were based on a calibration curve obtained with pyrogallol. The result was expressed as Pyrogallol equivalents (Pyr.) in milligrams per gram of extract.

The qRT-PCR was performed as in Luo *et al.* (2020) on three independent biological samples having three technical replications each. Six genes were selected for RNA-seq verification, and the primers used for qRT-PCR are shown in Supplementary Table 1. The relative gene expression was calculated using the 2^{-ΔΔCt} method, where β-actin was taken as the reference gene. The measured transcripts were normalized to the relative expression value of negative control.

Significant differences between treatments were determined by Duncan's Multiple Range Tests at P= 0.05 with SAS 8.1 (SAS Institute, Inc., Cary, NC, USA).

RESLUTS

Effects of Exogenous Phenolic Substances on the H₂O₂, O₂⁻ and MDA Content

The H₂O₂ content of the root increased in the positive control and caffeic acid treatment, while it also increased in the petiole of the positive control, coumaric acid, and ferulic acid treatments (Figure 1-a). The root O₂⁻ content of coumaric acid treatment was higher than others, and the petiole O₂⁻ content were promoted by *C. siamense* infection in positive and coumaric acid, caffeic acid, and ferulic acid treatments. The leaf O₂⁻ content were down-regulated by *C. siamense* infection in the positive and exogenous coumaric acid,

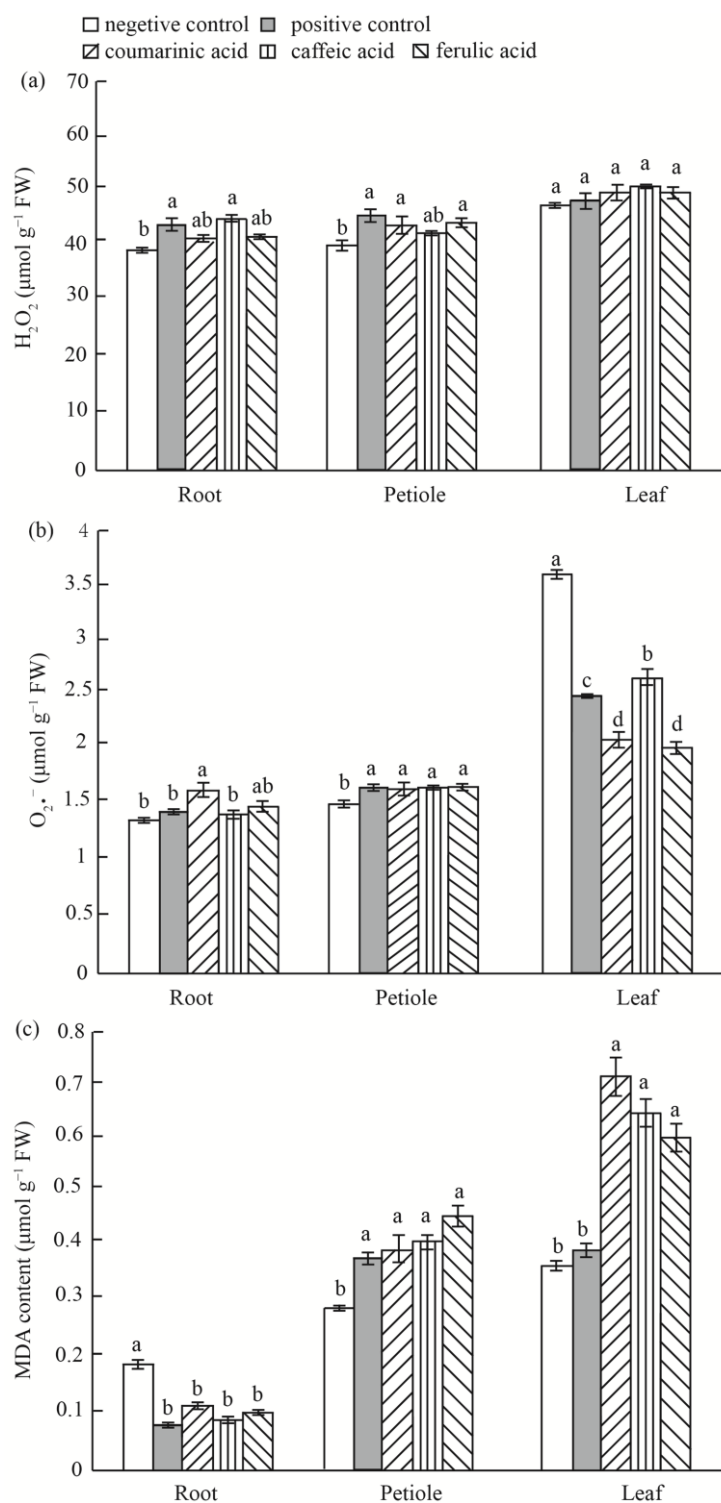


Figure 1. Effects of exogenous coumarinic acid, caffeic acid and ferulic acid on the H_2O_2 , $O_2^{\cdot-}$ and MDA content. Data (Means \pm SE, n= 6) followed by different letters above the bars indicate significant differences at the 5% level.

caffeic acid and ferulic acid treatments (Figure 1-b). The MDA content were decreased by *C. siamense* infection in positive and exogenous coumaric acid, caffeic acid and ferulic acid in root, but it showed opposite effects in petiole and leaf (Figure 1-c).

Effects of Exogenous Phenolic Substances on the SOD, CAT and POD Activities

The activities of SOD, CAT and POD showed different variation to *C. siamense* infection or phenolic acid treatment. The SOD activities of five treatments were nearly similar in root, and SOD activities of positive treatment, coumaric acid decreased in petiole and leaf (Figure 2-a). Compared with the negative control, the CAT activities of positive and caffeic acid treatments were significantly down-regulated. Compared with positive control, coumaric acid and ferulic acid promoted CAT activities in root. The CAT activities were promoted by coumaric acid and ferulic acid in petiole. The CAT activities were promoted by positive control and ferulic acid in leaf (Figure 2-b). The POD activities were all up-regulated in positive control, coumaric acid, caffeic acid, and ferulic acid treatments in root and petiole. The POD activities were up-regulated by caffeic acid and ferulic acid in leaf (Figure 2-c).

Effects of Exogenous Phenolic Substances on Total Phenolics and Soluble Protein Content

The total phenolics content was decreased by coumaric acid in root, but was up-regulated by coumaric acid in petiole. The total phenolics content in the negative control and caffeic acid treated leaf were significantly lower than that in the positive control and ferulic acid treatment (Figure 3-a). Compared with the negative control, other four treatments showed higher soluble

protein content, and the soluble protein content of three phenolics treatments was significantly higher than that of positive control in root and leaf. The soluble protein content of coumaric acid and ferulic acid treatments were higher than the negative and positive controls (Figure 3-b).

Effects of Exogenous Phenolic Substances on the Expression of Genes Involved in 'Phenylpropanoid Biosynthesis' Pathway in Petiole

The genes involved in 'phenylpropanoid biosynthesis' pathway were analyzed for their characteristic expression variation in phenolics treatments. The expression level of maker-Fvb7-2-augustus-gene-144.48, which encoded phenylalanine ammonia-lyase, was induced by *C. siamense* infection, caffeic acid, and ferulic acid, but was inhibited by coumaric acid. The transcript maker-Fvb4-1-augustus-gene-40.31, which encoded trans-cinnamate 4-monooxygenase (CYP73A), was induced in positive control and the three phenolics treatments, and the expression level was significantly higher in phenolics than that in the positive control. The transcript augustus_masked-Fvb6-4-processed-gene-231.3 and augustus_masked-Fvb6-1-processed-gene-149.7 encoding caffeoylshikimate esterase (GSE) showed different expression patterns. The augustus_masked-Fvb6-4-processed-gene-231.3 was inhibited by coumaric acid, but was induced by ferulic acid, while the augustus_masked-Fvb6-1-processed-gene-149.7 was induced by *C. siamense* infection and induced by coumaric acid. The transcripts maker-Fvb5-4-augustus-gene-52.49 and maker-Fvb5-4-augustus-gene-52.50 encoded Caffeic acid 3-O-Methyltransferase (COMT). The result showed that maker-Fvb5-4-augustus-gene-52.49 was up-regulated by caffeic acid and ferulic acid, while maker-Fvb5-4-augustus-gene-52.50 was up-regulated in the positive control and inhibited by the three phenolics (Figure 4).

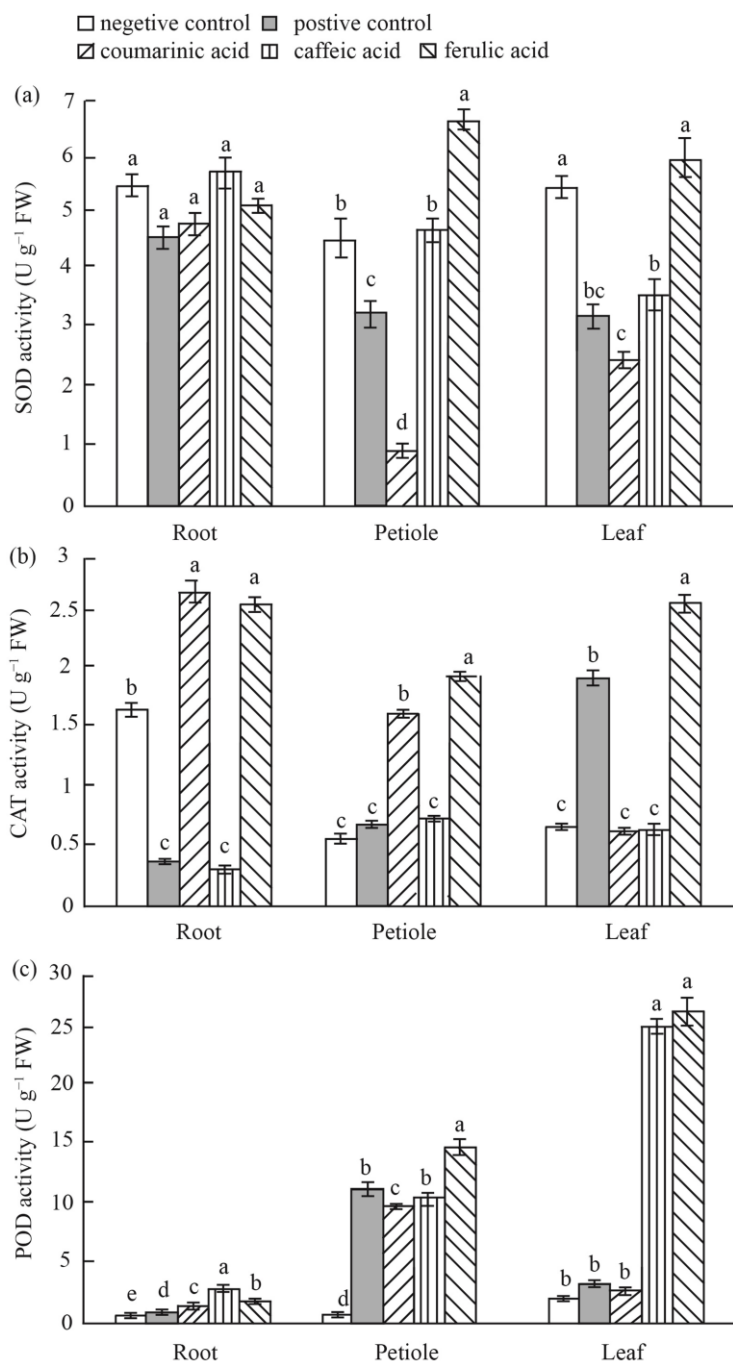


Figure 2. Effects of exogenous coumaric acid, caffeic acid and ferulic acid on the SOD, CAT and POD activities. Data (Means±SE, n= 6) followed by different letters above the bars indicate significant differences at the 5% level.

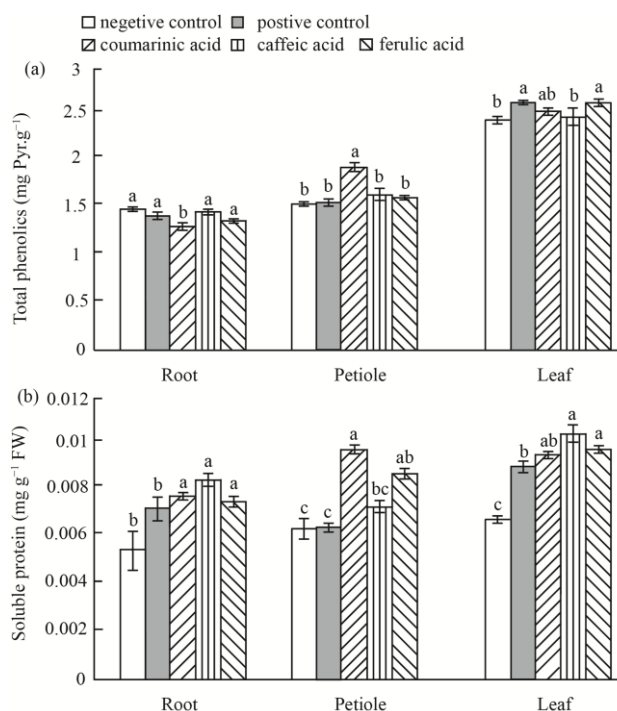


Figure 3. Effects of exogenous coumaric acid, caffeic acid, and ferulic acid on total phenolics and soluble protein content. Data (Means±SE, n= 6) followed by different letters above the bars indicate significant differences at the 5% level.

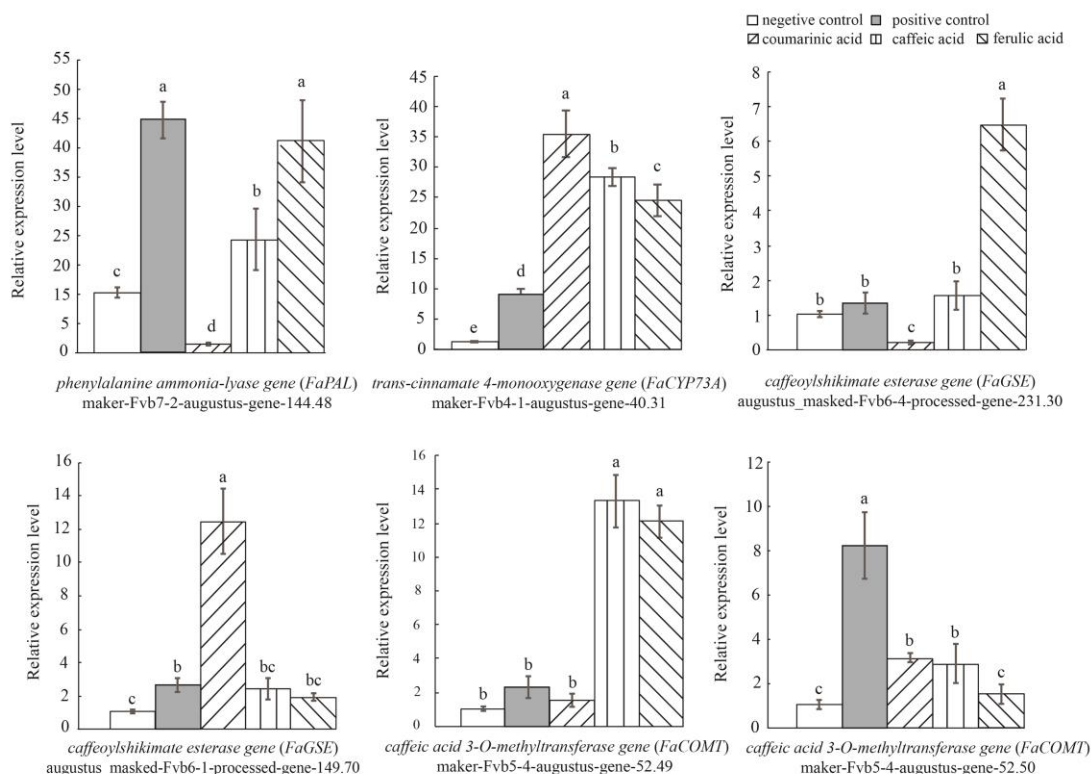


Figure 4. Effects of exogenous coumaric acid, caffeic acid and ferulic acid on the expression of genes involved in 'Phenylpropanoid biosynthesis' pathway in petiole. Data (Means±SE, n= 6) followed by different letters above the bars indicate significant differences at the 5% level.

Supplementary Table 1. Primer sequences used for qRT-PCR analysis.

Gene ID	Sequence of forward primer	Sequence of reverse primer
House-Keeping	AGCCTAACGCAGAGGGTTCCAAA	GCAGCCCCACATTGAAGGGTCTATAGT
Phenylalanine Ammonia-Lyase gene (<i>FaPAL</i>), maker-Fvb7-2-augustus-gene-144.48	TATTGCCGGGCTTTTGATCG	ATTCCGGGCCAACTTAAACGGC
<i>Trans</i> -cinnamate 4-monoxygenase gene (<i>FaCYP73A</i>), maker-Fvb4-1-augustus-gene-40.31	ATGCATGGTGGCTAGCAAAC	TCTTCCCTCAAAGAACCCTGCTCTG
Caffeoylshikimate esterase gene (<i>FaGSE</i>), augustus_masked-Fvb6-4-processed-gene-231.30	TCGACGGAAAAATCGGCATC	TGGGGGTTTTCCGAAGTAGGATTG
Caffeoylshikimate esterase gene (<i>FaGSE</i>), augustus_masked-Fvb6-1-processed-gene-149.70	AGGATAACTTCTCCCGCGTAAC	ACGCCTTCTCATACAACAGC
Caffeic acid 3-O-Methyltransferase gene (<i>FaCOMT</i>), maker-Fvb5-4-augustus-gene-52.49	TGCTTGATCGTATGCTTCGC	TACTTGCCTACCCGGTGCTAAAC
Caffeic acid 3-O-Methyltransferase gene (<i>FaCOMT</i>), maker-Fvb5-4-augustus-gene-52.50	AGCGGCCATAAAAACCTTGGTG	AGCATACGATCAAGCACTGC

DISCUSSION

ROS and POD Maintains the Balance in Process of Crown Rot in Petiole

H_2O_2 or O_2^- accumulated in the course of pathogen infection, and it was distinctive and closely followed the levels of disease resistance (Sharma *et al.*, 2012; Vuleta *et al.*, 2016). Although 'Benihoppe' strawberry is susceptible to crown rot caused by *C. siamense*, our results showed that H_2O_2 or O_2^- accumulated and increased the MDA content (Figure 1). Previous studies had demonstrated that the infection of wheat (*Triticum aestivum*) with *Septoria tritici* was accompanied by a large and early H_2O_2 accumulation during incompatible interactions, while very little H_2O_2 accumulated during the initial biotrophic phase of the interaction in a compatible interaction (Shetty *et al.*, 2003). Likewise, other studies had found that some biotrophic pathogens were inhibited by H_2O_2 , whereas necrotrophic pathogens were favored by H_2O_2 accumulation (Kumar *et al.*, 2011, Mellersh *et al.*, 2002). *C. siamense* infection led to strawberry death from aboveground in two weeks, which was taken as necrotrophic pathogen. Our result verified the evidence of *C. siamense* infection causing ROS acclimation upon necrotrophic pathogens infection (Figure 1). The H_2O_2 , O_2^- , and even MDA contents showed different responses to *C. siamense* infection in root, petiole, and leaf. The H_2O_2 , O_2^- and MDA were increased by *C. siamense* in petiole, but this did not occur in root and leaf. This might contribute to ROS triggered in local site for *C. siamense* infected petiole (Figure 1).

Besides repressed *C. siamense* SCR-7 infection, ROS targets are high-molecular mass molecules. Due to their positive and negative effects of ROS, it is accordingly necessary for the cells to control the level of ROS tightly to avoid any oxidative injury, and not to eliminate them completely (Sharma *et al.*, 2012). Scavenging or

detoxification of extra ROS could be also achieved through the activity of a complex network of antioxidant enzymes and non-enzymatic antioxidants (Dangl and Jones, 2001). Our study analyzed the activity of three main antioxidant enzymes, namely, SOD, CAT and POD and two non-antioxidant enzymes, total phenolics and soluble protein. The POD among the three antioxidant enzymes was significantly induced responding to *C. siamense* SCR-7 infection, which might suggest POD played the main role in this process (Figure 2).

Exogenous Phenolics Regulated the Expression of Genes Involved in Phenylpropanoid Biosynthesis

Compared with the negative control, *C. siamense* SCR-7 infection promoted the H_2O_2 , O_2^- and MDA in the petiole, and exogenous coumaric acid, caffeic acid, and ferulic acid promoted the H_2O_2 , O_2^- and MDA in the positive control (Figure 1), the coumaric acid down-regulated the POD activity but up-regulated total phenolics. The ferulic acid up-regulated POD activity but not the total phenolics (Figures 2 and 3). In addition to ROS, POD can catalyze oxidation of phenolics to form quinines, which was considered to be important in the phenylpropanoid biosynthesis process (Ma *et al.*, 2022). POD activity was up-regulated, which might suggest exogenous coumaric acid, caffeic acid and ferulic acid increased susceptibility of *C. siamense* causing crown rot by regulating the ROS triggering phenylpropanoid biosynthesis.

Phenylpropanoid biosynthesis is a complex network regulated by multiple gene families such as Phenylalanine AmmoniaL (PAL) catalyzing the conversion of phenylalanine to cinnamic acid, CYP73A catalyzing the conversion of cinnamic acid to coumaric acid (Yadav *et al.*, 2020). In addition, coumaric acid is further hydroxylated to caffeic acid, and then converted into ferulic acid and sinapic acid under the catalysis of GSE and COMT.



Normally, the catalytic reaction was promoted by incensement of substrate concentration and inhibited by reaction products (Rodrigues *et al.*, 2013). Our experiment showed *FaCYP73A* (maker-Fvb4-1-augustus-gene-40.31) was inhibited by coumaric acid products but induced by the three phenolics. However, the *FaGSE* (augustus_masked-Fvb6-4-processed-gene-231.3 and augustus_masked-Fvb6-1-processed-gene-149.7) were inhibited or induced by coumaric acid and showed no difference after caffeic acid treatment. Only the *FaCOMT* transcripts (maker-Fvb5-4-augustus-gene-52.49 and maker-Fvb5-4-augustus-gene-52.50) were up-regulated by caffeic acid and ferulic acid (Figure 4). This suggested that the regulation process was more complex than catalytic reaction. Recent study showed that the phenylpropanoid pathway inhibitor piperonylic acid induces broad-spectrum disease resistance in plants by reprogramming phenylpropanoid metabolism and locally affecting reactive oxygen species metabolism (Desmedt *et al.* 2021).

CONCLUSIONS

Exogenous coumaric acid, caffeic, acid and ferulic acid were used to explore whether metabolites involved in phenylpropanoid biosynthesis increased susceptibility to crown rot associated with reactive oxygen species, antioxidant substance and key genes of phenylpropanoid metabolism regulation. According to the results, H₂O₂, O₂⁻ and MDA contents showed different responses to *C. siamense* infection in root, petiole and leaf, and H₂O₂, O₂⁻ and MDA were increased by *C. siamense* in petiole. It is more accurate to evaluate crown rot with petiole than with leaf. Exogenous coumaric acid, caffeic acid, and ferulic acid promoted the H₂O₂, O₂⁻ and MDA in the positive control. The POD activity was significantly induced responding to *C. siamense* infection in

petiole than other enzymes and non-antioxidant enzymes, suggesting that POD plays the main role in this process. The coumaric acid treatment down-regulated the POD activity, but increased total phenolics, while the ferulic acid only up-regulated POD activity in petiole. In addition, the expressions of transcripts involved in phenylpropanoid biosynthesis, such as *FaCYP73A*, *FaGSE* and *FaCOMT* transcripts were regulated by exogenous coumaric acid, caffeic acid, and ferulic acid.

REFERENCES

1. Camejo, D., Martí, M. C., Jiménez, A., Cabrera, J. C., Olmos, E. and Sevilla, F. 2011. Effect of Oligogalacturonides on Root Length, Extracellular Alkalinization and O₂⁻-Accumulation in Alfalfa. *J. Plant Physiol.*, **168**: 566–575.
2. Dangl, J. L. and Jones, J. D. 2001. Plant Pathogens and Integrated Defense Responses to Infection. *Nature*, **411**: 826–833.
3. de Freitas-Silva, L., Rodríguez-Ruiz, M., Houmani, H., da Silva, L. C., Palma, J. M. and Corpas, F. J. 2017. Glyphosate-Induced Oxidative Stress in *Arabidopsis thaliana* Affecting Peroxisomal Metabolism and Triggers Activity in the Oxidative Phase of the Pentose Phosphate Pathway (OxPPP) Involved in NADPH Generation. *J. Plant Physiol.*, **218**: 196–205.
4. Deng, J., Bi, Y., Zhang, Z., Xie, D., Ge, Y., Li, W., Wang, J. and Wang, Y. 2015. Postharvest Oxalic Acid Treatment Induces Resistance against Pink Rot by Priming in Muskmelon (*Cucumis melo* L.) Fruit. *Postharvest Biol. Technol.* **106**: 53–61.
5. Desmedt, W., Jonckheere, W., Nguyen, V. H., Ameye, M., De Zutter, N., De Kock, K., Debode, J., Van Leeuwen, T., Audenaert, K., Vanholme, B. and Kyndt, T. 2021. The Phenylpropanoid Pathway Inhibitor Piperonylic Acid Induces Broad-Spectrum Pest and Disease Resistance in Plants. *Plant Cell Environ.*, **44**: 3122–3139
6. Díaz-Vivancos, P., Barba-Espín, G., Clemente-Moreno, M. J. and Hernández, J. A. 2010. Characterization of the Antioxidant System during the Vegetative

- Development of Pea Plants. *Biol. Plantarum*, **54**: 76-82.
7. Gilroy, S., Suzuki, N., Miller, G., Choi, W. G., Toyota, M., Devireddy, A. R. and Mittler, R. 2014. A Tidal Wave of Signals: Calcium and ROS at the Forefront of Rapid Systemic Signaling. *Trends Plant Sci.*, **19**: 623–630.
 8. He, J. D., Zou, Y. N., Wu, Q. S. and Kuča, K. 2020. Mycorrhizas Enhance Drought Tolerance of Trifoliolate Orange by Enhancing Activities and Gene Expression of Antioxidant Enzymes. *Sci. Hort.-AMSTERDAM*, **262**: 108745.
 9. Kumar, N., Prabhu, P. A. J., Pal, A. K., Remya, S., Aklakur, M., Rana, R. S., Gupta, S., Raman R. P. and Jadhao, S. B. 2011. Anti-Oxidative and Immunohematological Status of Tilapia (*Oreochromis mossambicus*) during Acute Toxicity Test of Endosulfan. *Pestic. Biochem. Phys.*, **99**: 45–52.
 10. Li, G., Zhu, S., Wu, W., Zhang, C., Peng, Y., Wang, Q. and Shi, J. 2017. Exogenous Nitric Oxide Induces Disease Resistance Against *Monilinia fructicola* through Activating the phenylpropanoid Pathway in Peach Fruit. *J. Sci. Food Agric.*, **97**: 3030–3038.
 11. Li, X., Lin, J., Gao, Y., Han, W. and Chen, D. 2012. Antioxidant Activity and Mechanism of Rhizoma Cimicifugae. *Chem. Cent. J.*, **6**: 1–10.
 12. Luo, C., Hu, Y. Y., and Shu, B. 2021. Characterization of *Colletotrichum siamense* Causing Crown Rot of Strawberry in Jingzhou, Hubei Province. *Not. Bot. Horti. Agrobo.*, **49**: 1–13.
 13. Luo, C., Sun, Q., Zhang, F., Zhang, D., Liu, C., Wu, Q., and Shu, B. 2020. Genome-Wide Identification and Expression Analysis of the Citrus Malectin Domain-Containing Receptor-Like Kinases in Response to Arbuscular Mycorrhizal Fungi Colonization and Drought. *Hortic. Environ. Biote.*, **61**: 891–901.
 14. Ma, W., Li, J., Murtaza, A., Iqbal, A., Zhang, J., Zhu, L., Xu, X. Pan, S., and Hu, W. 2022. High-Pressure Carbon Dioxide Treatment Alleviates Browning Development by Regulating Membrane Lipid Metabolism in Fresh-Cut Lettuce. *Food Control*, **134**: 108749.
 15. Matern, U., and Kneusel, R.E., 1988. Phenolic Compounds in Plant Disease Resistance. *Phytoparasitica*, **16**: 153–170.
 16. Mellersh, D. G., Foulds, I. V., Higgins, V. J., and Heath, M. C. 2002. H₂O₂ Plays Different Roles in Determining Penetration Failure in Three Diverse Plant–Fungal Interactions. *Plant J.*, **29**: 257–268.
 17. Nath, M., Bhatt, D., Prasad, R., and Tuteja, N. 2017. Reactive Oxygen Species (ROS) Metabolism and Signaling in Plant-Mycorrhizal Association under Biotic and Abiotic Stress Conditions. In: “*Mycorrhiza Eco-Physiology, Secondary Metabolites, Nanomaterials*”, (Eds.): Varma, A., Prasad, R. and Tuteja, N. Springer, Cham, PP. 223–232.
 18. Nathan, C. and Cunningham-Bussel, A. 2013. Beyond Oxidative Stress: An Immunologist's Guide to Reactive Oxygen Species. *Nat. Rev. Immunol.*, **13**: 349–361.
 19. Oliveira, M. D. M., Varanda, C. M. R. and Félix, M. R. F. 2016. Induced Resistance during the Interaction Pathogen X Plant and the Use of Resistance Inducers. *Phytochem. Lett.*, **15**: 152–158.
 20. Ray, P. D., Huang, B. W. and Tsuji, Y. 2012. Reactive Oxygen Species (ROS) Homeostasis and Redox Regulation in Cellular Signaling. *Cell Signal*, **24**: 981–990.
 21. Rodrigues, R. C., Ortiz, C., Berenguer-Murcia, Á., Torres, R. and Fernández-Lafuente, R. 2013. Modifying Enzyme Activity and Selectivity by Immobilization. *Che. Soc. Rev.*, **42**: 6290–6307.
 22. Sharma, P., Jha, A. B., Dubey, R. S. and Pessarakli, M. 2012. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *J. Botany*, **2012**: 217037
 23. Shetty, N. P., Kristensen, B. K., Newman, M. A., Møller, K., Gregersen, P. L. and Jørgensen, H. L. 2003. Association of Hydrogen Peroxide with Restriction of *Septoria tritici* in Resistant Wheat. *Physiol. Mol. Plant P.*, **62**: 333–346.
 24. Sudhakar, C., Lakshmi, A. and Giridarakumar, S. 2001. Changes in the Antioxidant Enzyme Efficacy in Two High Yielding Genotypes of Mulberry (*Morus alba* L.) under NaCl Salinity. *Plant Sci.*, **161**: 613–619.



25. Suzuki, N., Miller, G., Morales, J., Shulaev, V., Torres, M. A. and Mittler, R. 2011. Respiratory Burst Oxidases: the Engines of ROS Signaling. *Curr. Opin. Plant Biol.*, **14**: 691–699.
26. Velikova, V., Yordanov, I. and Edreva, A. 2000. Oxidative Stress and Some Antioxidant Systems in Acid Rain-Treated Bean Plants: Protective Role of Exogenous Polyamines. *Plant Sci.*, **151**: 59–66.
27. Vivancos, P. D., Dong, Y., Ziegler, K., Markovic, J., Pallardó, F. V., Pellny, T. K., Verrier, P. J. and Foyer, C. H. 2010. Recruitment of Glutathione into the Nucleus during Cell Proliferation Adjusts Whole-Cell Redox Homeostasis in *Arabidopsis thaliana* and Lowers the Oxidative Defence Shield. *Plant J.*, **64**: 825–838.
28. Vuleta, A., Jovanović, S. M. and Tucić, B. 2016. Adaptive Flexibility of Enzymatic Antioxidants SOD, APX and CAT to High Light STress: The Clonal Perennial Monocot Iris *Pumila* as a Study Case. *Plant Physiol. Bioch.*, **100**: 166–173.
29. Wu, Q.S. 2018. Experimental Guidelines in Plant Physiology. China Agriculture Press, Beijing, China, pp. 1–171
30. Wu, Q. S., Zou, Y. N. and Xia, R. X. 2006. Effects of Water Stress and Arbuscular Mycorrhizal Fungi on Reactive Oxygen Metabolism and Antioxidant Production by Citrus (*Citrus tangerine*) Roots. *Eur. J. Soil Biol.*, **42**: 166–172.
31. Yadav, V., Wang, Z., Wei, C., Amo, A., Ahmed, B., Yang, X. and Zhang, X. 2020. Phenylpropanoid Pathway Engineering: An Emerging Approach towards Plant Defense. *Pathogens*, **9**: 312.
32. Yong-Hong, G. E., Yang, B. I., Li, Y. C. and Wang, Y. 2012. Resistance of Harvested Fruits and Vegetables to Diseases Induced by ASM and Its Mechanism. *Sci. Agri. Sinica.*, **16**: 3357–3362.
33. Zhang, M., Wang, D., Gao, X., Yue, Z. and Zhou, H. 2020. Exogenous Caffeic Acid and Epicatechin Enhance Resistance against *Botrytis cinerea* through Activation of the Phenylpropanoid Pathway in Apples. *Sci. Hortic. AMSTERDAM*, **268**: 109348.
34. Zhu, J. X., Wu, H. and Sun, Q. J. 2019. Preparation of Crosslinked Active Bilayer Film Based on Chitosan and Alginate for Regulating Ascorbate-Glutathione Cycle of Postharvest Cherry Tomato (*Lycopersicon esculentum*). *Int. J. Biol. Macromol.*, **130**: 584–594.

تأثیر مواد فنلی خارجی (Exogenous) بر حساسیت توت فرنگی اکتوپلوئید به پوسیدگی طوقه

چ. لو، ی. هو، و. ب. شو

چکیده

پوسیدگی طوقه ناشی از حمله *Colletotrichum siamense* یک بیماری جدی توت فرنگی در منطقه رودخانه یانگ تسه (Yangtze) چین است. متابولیت های مسول زیست ساخت فنیل پروپانویید (phenylpropanoid) باعث افزایش حساسیت به پوسیدگی طوقه در توت فرنگی اکتوپلوئید (octoploid) می شود. برای بررسی اینکه آیا افزایش حساسیت با گونه های فعال اکسیژن، ماده آنتی اکسیدانی و ژن های کلیدی متابولیسم فنیل پروپانویید مرتبط است یا خیر، در این پژوهش، اسید کوماریک اگزوزن، اسید کافئیک و اسید

فرولیک درگیر در زیست‌ساخت فنیل پروپانویید استفاده شد. با توجه به نتایج، محتوای H_2O_2 ، $\text{O}_2^{\cdot-}$ و MDA واکنش‌های متفاوتی به عفونت *C. siamense* در ریشه، دم‌برگ و برگ نشان داد. مقدار H_2O_2 ، $\text{O}_2^{\cdot-}$ و MDA توسط *C. siamense* در دم‌برگ افزایش یافت. اسید کوماریک، اسید کافئیک و اسید فرولیک خارجی مقدار H_2O_2 ، $\text{O}_2^{\cdot-}$ و MDA را در تیمار شاهد مثبت بهبود بخشید. در پاسخ به عفونت *C. siamense*، فعالیت POD به طور قابل توجهی در دم‌برگ القا شد. تیمار اسید کوماریک فعالیت POD را مهار کرد اما فنل کل را افزایش داد. اسید فرولیک فقط فعالیت POD را در دم‌برگ ارتقا داد. افزون بر این، بیان رونوشت‌های دخیل در زیست‌ساخت فنیل پروپانویید توسط اسید کوماریک، اسید کافئیک و اسید فرولیک تنظیم شد. به طور کلی، نتایج ما نشان داد که کاربرد اسید کوماریک، اسید کافئیک و اسید فرولیک حساسیت توت‌فرنگی اکتوپلوئید به پوسیدگی طوقه را از طریق ROS تنظیم شده، ماده آنتی‌اکسیدانی و بیان رونوشت‌های زیست‌ساخت فنیل پروپانویید افزایش می‌دهد.