Postharvest Biology and Technology 145 (2018) 61–73

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Comparative metabolomics and transcriptomic profiling reveal the mechanism of fruit quality deterioration and the resistance of citrus fruit against *Penicillium digitatum*

Ning Tang[a,b,c], Nan Chen[a], Nan Hu[a], Wei Deng[a], Zexiong Chen[b,c,**], Zhengguo Li[a,c,*]

[a] School of Life Sciences, Chongqing University, Chongqing, 400030, China
[b] Research Institute for Special Plants, Chongqing University of Arts and Sciences, Chongqing, 402160, China
[c] Collaborative Innovation Center of Special Plant Industry in Chongqing, Chongqing, 400000, China

ARTICLE INFO

Keywords:
Citrus fruit
Penicillium digitatum
Metabolome
Transcriptomic analysis

ABSTRACT

Decay, caused by *Penicillium* infection, is one of the main problems during postharvest storage in citrus fruit. A deeper understanding of the mechanism of fruit quality deterioration and the fruit's defense responses may lead us to develop rational approaches for disease control. To identify the metabolic and regulatory mechanisms of *citrus* against *Penicillium digitatum*, Powell orange (*Citrus sinensis*) fruit pulps were collected after 40 h and 60 h infected with *P. digitatum* for RNA-Seq analysis complemented with metabolic profiling of polar metabolites and volatile emissions. Results demonstrated that several primary metabolites and volatile organic compounds (VOCs) were significantly regulated in response to *P. digitatum* infection. The contents of sugars (e.g. glucose and sucrose), organic acids (e.g. citric acid, malic acid, and oxalic acid), vitamin C and D-limonene were decreased, while others such as ethanol and a-terpineol were in outstanding increases, resulting in fruit quality deterioration. Rhamnose, inositol, serine, threonine and γ-aminobutyric acid (GABA) also accumulated, thereby enhancing the resistance to *P. digitatum* infection. RNA-Seq data revealed a series of significantly enriched pathways. *P. digitatum* infection induced G-protein and RLK signal pathways and enhanced the transcription of stress-related genes including peroxidase and NBS-LRR. Furthermore, *P. digitatum* infection triggered a defense response via both the jasmonic acid and ethylene pathways, and increased the transcript abundance of several transcription factors such as ERFs, WRKYs, and MYB. Phenylpropanoids pathways were also activated at the transcriptomic level. In summary, our results unravel the significantly regulated metabolites and biological pathways, which provide new insights into the mechanism of fruit quality deterioration and the induction of resistance against *P. digitatum* in Powell fruit.

1. Introduction

Citrus is the largest fruit crop worldwide with a global production of approximately 178 million metric tons in 2015 (Food and Agriculture Organization Corporate Statistical Database, FAO, http://faostat.fao.org). Citrus fruit have various types of metabolites including sugars, organic acids, amino acids, volatiles, phenolics, carotenoids and vitamins, which are essential factors responsible for their nutritional and health benefits (Cerdan-Calero et al., 2012). Although large amounts of citrus fruit are used to produce juice, they are primarily utilized for fresh consumption in markets in China. During postharvest handling and storage, citrus fruit are susceptible to pathogens. *Penicillium digitatum* and *Penicillium italicum* are responsible for the most serious diseases in citrus fruit, causing metabolic alterations and consequently loss in fruit quality and decay (Palou, 2013). Accordingly, fruit have developed a range of defense mechanisms against pathogen attack via changes in several volatiles and primary metabolites. Hence, knowledge about metabolites profiling is of major importance for biomarkers discovery during pathogen infection, which has remarkable implications for our food supply, nutrition, and health, and also provides insights into effective strategies for fruit quality control.

Previous studies deal with global metabolomic profiling involved in the enhancement of disease resistance in citrus fruit mediated by *Rhodosporidium paludigenum* (Lu et al., 2015) and heat treatment (Yun et al., 2013). A series of primary metabolites, such as ornithine, oleic acid, and tetradecanoic, and flavonoids including hesperetin,

* Corresponding author at: School of Life Sciences, Chongqing University, Chongqing, 400030, China.
** Corresponding author at: Research Institute for Special Plants, Chongqing University of Arts and Sciences, Chongqing, 402160, China.
E-mail addresses: sabrina-0810@hotmail.com (N. Tang), chennan0205@gmail.com (N. Chen), hunan.1210@163.com (N. Hu), dengwei1977@cqu.edu.cn (W. Deng), chenzexiong1979@163.com (Z. Chen), zhengguoli@cqu.edu.cn (Z. Li).

https://doi.org/10.1016/j.postharvbio.2018.06.007
Received 27 March 2018; Received in revised form 25 June 2018; Accepted 28 June 2018
0925-5214/ © 2018 Elsevier B.V. All rights reserved.

naringenin, diosmin, and rutin is up-regulated in heat- treated citrus pericarp (Yun et al., 2013). Lignin content increases in citrus peels subjected to heat and *Rhodosporidium* treatment (Lu et al., 2015; Yun et al., 2013). These findings indicated that the formation of physical defensive barriers and the accumulation of antimicrobial compounds in fruit peels are two types of resistance mechanisms. Recently, Ballester et al. (2013a, 2013b) reported significant changes in the metabolites of citrus peels infected with *P. digitatum* through HPLC/HPLC-QTOF-MS. They observed that several members of phenylpropanoids and flavonoids, especially coumarin scoparone, citrusnin A, and drupanin aldehyde are induced in citrus fruit, which are known to exert antimicrobial activity and act as phytoalexins in the defense response of citrus fruit against pathogen attack (Kim et al., 2011). However, the identified metabolites involved in resistance against *P. digitatum* are limited. Complete metabolomic profiling has yet to provide details about the changes in *P. digitatum*-infected citrus fruit.

These dynamic metabolic processes involve a series of molecular changes under gene regulation in response to *P. digitatum* infection. González-Candelas et al. (2010) conducted a subtracted cDNA library screening and revealed that induction of secondary and amino acid metabolisms constitutes the major response of citrus fruit to *P. digitatum* infection. A large subset of genes associated with stress response and phenylpropanoid biosynthetic process is activated in the flavedo when citrus fruit were exposed to *P. digitatum* infection, including *chitinase* (CHI1), *PR10A*, *phenylalanine ammonia-lyase* (PAL1), *cinnamate 4-hydroxylase* (C4H1), *isoflavone reductase* (IRL1), *caffeic acid 3-O-methyltransferase* (COMT1), *caffeoyl-CoA O-methyltransferases* (CCoAOMT1 and CCoAOMT2), *cinnamyl alcohol dehydrogenases* (CAD2 and CAD3), *sinapyl alcohol dehydrogenase* (SAD), *peroxidase* (POX1), and *leucoanthocyanidin dioxygenase-like protein* (LDOX) (Ballester et al., 2011, 2013b). Furthermore, large amounts of evidence has been presented that plant hormones, such as ethylene, jasmonates (JA) and salicylic acid (SA), play a pivotal role in promoting host resistance in citrus, (Dalio et al., 2017). Huanglongbing(HLB) strongly induces genes involved in ethylene biosynthesis and signal transduction, such as *ACO4*, *ETR1*, *ERF1* and *ERF2*. SA and JA pathways were also activated in CaLas-infected citrus (Martinelli et al., 2012). Gomez-Munoz et al. (2017) observed that the resistance of sour orange to Citrus tristeza virus (CTV) is mediated by SA defense pathways. Silencing of *RDR1*, *NPR1* and *DCL2/DCL4*, genes associated with these defense pathways, enhances virus spread and accumulation in sour orange plants. SA is also involved in the local defense response induced by *Xanthomonas citri* ssp. *citri* (*X. citri*), and the genes involved in SA biosynthesis, signaling, and response are up-regulated (Roeschlin et al., 2017). Moreover, exogenous application of SA and JA can significantly alleviate the severity and lesion diameter of canker disease caused by *X. citri* (Nayem et al., 2017). Recently, Ballester et al (2011) demonstrated that ethylene plays an important role in triggering induced resistance of citrus fruit in response to *P. digitatum* infection. However, limited information regarding the involvements of other hormone pathways and factors in the resistance of citrus fruit to *P. digitatum* infection is available.

Although elaborate research has been performed, the biochemical and molecular basis of resistance to *P. digitatum* remains partially elucidated. Unravelling the complexity of the mechanisms responsible for fruit susceptibility to *P. digitatum* infection and their effects on fruit characteristics is critically important to improve fruit quality in global markets with limited use of fungicides. In the present study, a system analysis via transcriptomics and metabolomics were employed to characterize the dynamic changes in the metabolites and fundamental regulatory pathways of Powell orange fruit [*Citrus sinensis* (L.) Osb.] infected with *P. digitatum*. This work brings novel insights into how fungal diseases, particularly *P. digitatum*, may affect host metabolism and the regulation of postharvest fruit quality. Furthermore, the initiation of defense mechanisms against pathogens has been illustrated.

2. Materials and methods

2.1. Plant materials and P. digitatum infection

Powell orange fruit at the commercial ripening stage were collected from eight-year-old “Powell”orange trees on red tangerine (Citrus reticulata Blanco) rootstock, which were planted in a local orchard located at Tiefo of Fengjie County, Chongqing, China. The fruit with a uniform size and color were selected from 20 individual trees, surface sterilized and dried.

P. digitatum was obtained from decayed citrus fruit and maintained in potato dextrose agar (PDA) at 5 °C. Conidia production was achieved by exposing inoculated Petri dishes with PDA to continuous dark, at 25 °C. The conidia were harvested from 7-day-old cultures, flooded and resuspended in sterile distilled water containing 0.05% (V/V) Tween 80. The spore concentration was adjusted to 10^6 conidia mL^{-1}.

The fruit were divided into two groups. In one group, fruit at harvest (day 0) without treatment was set as control (Fig. S1). And the other group was inoculated with *P. digitatum* spore suspension. Four wounds (2 mm deep and 5 mm diameter) were made with a sharp blade in the equatorial axis of each fruit. 20 μL of *P. digitatum* spore suspension was applied to each wound. Then, the fruit samples were stored at 25 °C and 90%–95% relative humidity. For each time point and each treatment, three biological replicates with 10 fruit samples per replicate were performed. After 40 h of inoculation, a little white mycelium was presented in flavedo tissues around the inoculation point. After 60 h of incubation, we observed that wet rot occurred in approximately one third of the flavedo surface in all the fruits infected by *P. digitatum*. Control and infected fruit at these two time points were collected, washed and sliced into halves longitudinally, and then juice sacs and juices were obtained from each section. Juices were collected using a manual extractor, sieved through gauze. All the samples were frozen in liquid nitrogen immediately and stored at -80 °C until analysis.

2.2. GC–MS analysis of polar metabolites

The profiling of polar metabolites was performed as detailed previously (Tang et al., 2016). 400 μL of juice was transferred into a glass vial and dried by nitrogen blowing instrument. 80 μL methoxamine hydrochloride solutions (15 g L^{-1}, in pyridine) were emptied into the dried samples and then the glass vial was vortexed and incubated at 37 °C for 90 min. For trimethylsilylation, 80 μL of BSTFA (with 1% TMCS) was poured in the glass vial and heated at 70 °C for 1 h. The resulting mixture was used as a GC–MS analysis sample. The polar metabolites were determined in six replicates.

GC–MS was performed using an Agilent 7890 A GC/5975C MS system, equipped with an Agilent J&W Scientific HP-5MS capillary column (5% phenyl/95% methylpolysiloxane, 30 m × 250 μm i.d.) under the following parameters:oven temperature ramp at 70 °C for 2 min, 10 °C min^{-1} ramp up to 140 °C, then 4 °C min^{-1} ramp up to 240 °C, finally heating at 10 °C min^{-1} until 300 °C and held for 8 min. Helium was used as a carrier gas at a constant flow of 1.0 mL min^{-1}. The injector temperature was held at 270 °C and a volume of 1 μL was injected in a splitless mode. The EI ion source temperature was kept at 230 °C and the quadrupole temperature was kept at 150 °C. Mass spectra and TIC were acquired over the mass range *m/z* 50 - 600.

The resulting high-throughput chromatography data files were typically processed via R software (http://cran.r-project.org/) and TagFinder to identify and quantify the metabolites. Relative abundance was determined on the basis of the normalized peak area. Principal Component Analysis (PCA) analysis was conducted using SIMCA-P version 11 (Umetrics, Umea,Sweden). To identify the differentially expressed metabolites, ANOVA and variable influence on projection (VIP) values were employed to determine statistical significance. Differences were considered significant when $P < 0.05$ and VIP > 1. Finally, the compound was identified by comparing its MS with those

Download English Version:

https://daneshyari.com/en/article/8881769

Download Persian Version:

https://daneshyari.com/article/8881769

Daneshyari.com