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Chlorogenic acid induces resistance against *Penicillium expansum* in peach fruit by activating the salicylic acid signaling pathway

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ABSTRACT

The objective of this study was to investigate the effect of chlorogenic acid (CGA) treatment on induction of resistance against pathogens in peach fruit. Treatment with CGA at 25–150 mg L^{-1} was effective at reducing lesion diameter and decay index of peach fruit during storage at 25 °C after *Penicillium expansum* infection. CGA treatment had also significant beneficial effects on fruit quality parameters including firmness, soluble solids contents, pH value, and titratable acidity. Activities of main defense-related enzymes and expression of key genes involved in the salicylic acid (SA) signaling pathway, such as *PAL*, *ICS*, *WRKY*, *NPR1*, *PR1*, *CHI*, *GLU*, *PR5*, and *POD*, were enhanced by CGA treatment. These findings suggest that CGA treatment is a promising approach to controlling postharvest blue mold rot in peach fruit, and activation of the SA signaling pathway may play a potential role in mechanisms involving CGA-induced plant disease resistance.

1. Introduction

Peach (*Prunus persica* L.) fruit, belongs to climacteric fruit, has been one of the most popular fresh fruits in the world. However, it has only a short shelf life after harvest when stored at ambient temperature because of its high susceptibility to pathogens. Blue mold rot caused by *P. expansum* is one of the major postharvest diseases in fruit, and leads to considerable economic losses (Zhu, Yu, Brecht, Jiang & Zheng, 2015). Synthetic fungicides have been applied extensively in the past to control postharvest diseases in fruit and vegetables, because they are cheap and effective. Negative effects of synthetic fungicides, such as environmental pollution, health risks, and pathogen resistance, have stimulated more and more studies seeking other approaches to controlling postharvest diseases in fruit. Boosting natural disease resistance (NDR) in fruit is considered an efficient and preferred strategy for reducing disease incidence (Terry & Joyce, 2004). It is now well documented that NDR is systemically and directly triggered by a variety of abiotic and biotic inducers including physical, chemical, or biological elicitors (Wang, Cao, Jin, Rui & Zheng, 2010; Wang, Jin, et al., 2014).

In plant defense systems, systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two predominant forms of inducing disease resistance. Both SAR and ISR can be induced against a broad spectrum of pathogenic fungi and bacteria with partly overlapping effects (van Loon, Rep & Pieterse, 2006). Induction of SAR is a promising approach to utilizing the host's natural disease resistance for controlling postharvest diseases. SAR promotes broad-spectrum protection against pathogens mediated through salicylic acid (SA) biosynthesis induction, SA signaling pathway activation, and production of pathogenesis-related proteins (PRs) (Sun, Zhang, Guo, Yu & Chen, 2013). PRs possess active defenses and participate in suppressing pathogen development and spread in plants (Liu et al., 2016). In particular, expression of a *PR-1* or accumulation of the corresponding protein is usually taken as a hallmark of SAR activation. SA can elicit all *PR-1* genes in most plants. Activation of the SA signaling pathway is essential for the onset of SAR in plants (van Loon et al., 2006; Vernooij et al., 1994).

Chlorogenic acid (CGA, *3-O*-caffeoylquinic acid), is an important phenolic compound belonging to the hydroxycinnamic acid derivative family and consists of caffeic acid and quinic acid through esterification. (Santanagálvez, Cisneroszevallos & Jacobovelázquez, 2017). At present, it is studied extensively for its antioxidant properties, and is a promising bioactive compound used as a dietary supplement, food preservative, and a functional food. Overwhelming epidemiological studies, animal tests and clinical trials have demonstrated that CGA exerts various health-promoting properties including anti-inflammatory effects (Liang & Kitts, 2016), antidiabetic activity (Bagdas et al., 2015), reduction of blood pressure (Suzuki et al., 2006), neuroprotective effects (Kim et al., 2012), and alleviation of cardio-vascular disease risks (Zhao, Bomser, Joseph & Disilvestro, 2013). In addition, there may be a close relationship between the level of CGA content and defense responses in plants. It has been reported that more resistant plant varieties have higher levels of CGA (Wang, Li, et al., 2014). Moreover,

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Atanasova-Penichon et al. (2012) has found that CGA is the main free phenolic acid, which plays a role in maize against *Fusarium graminearum* and trichothecene accumulation. Nevertheless, there are still large gaps in our knowledge concerning the effect of exogenous CGA on plant disease resistance.

In our previous study, it was found that postharvest treatment with CGA maintained postharvest quality and enhanced the antioxidant capacity of nectarine fruit (Xi, Jiao, Cao & Jiang, 2017; Xi, et al., 2017). Accordingly, the purpose of this study was to evaluate the effect of CGA on blue mold rot in peach during storage and provide an insight into its mechanism of action involved. The results indicated for the first time, that CGA can be used as a prospective chemical elicitor for controlling blue mold rot in peach, and activation of the SA signaling pathway may play a potential role in mechanisms involving CGA-induced plant disease resistance. This work is meaningful because CGA is easily obtainable from several agroindustrial by-products which are rich in this compound. (Martínez, et al., 2017).

2. Materials and methods

2.1. Fruits materials, fungal cultures and CGA

Peach fruit (*Prunus persica* L. cv. gangshanhong) were harvested at commercial maturity from an experimental orchard in Beijing, China and immediately delivered to our laboratory. Fruit with uniformity in shape, color, size and without pathogens infection or physical injury were selected for the following experiments. Then, all fruit were surface-disinfected with 0.05% (v/v) sodium hypochlorite for 2 min, washed with tap water, and air-dried at room temperature.

The strain of *P. expansum* isolated from peach fruit occurred blue mold rot was maintained on potato dextrose agar (PDA) and cultured for 7 d at 28 °C. The spore suspension was prepared by washing the cultures with sterile distilled water. After filtering through four layers of sterile gauze, a spore suspension without any adhering mycelia was collected for determining spore concentration with the aid of a hemocytometer. Finally, a spore suspension at 1×10^4 spores mL^{-1} was obtained for the experiments.

CGA (PubChem CID: 1794427) was purchased from Fluka-Sigma-Aldrich (St. Louis, MO, USA). The powder of CGA was diluted in sterile distilled water to get various concentrations (0, 25, 50, 100, 150 mg L^{-1}).

2.2. Effect of CGA concentration on controlling blue mold rot caused by P. expansum in peach fruit

Peach fruit were infiltrated with CGA at different concentrations (0, 25, 50, 100, and 150 mg L^{-1}) under vacuum (−0.02 MPa) for 2 min, and then air-dried at room temperature. Twenty-four hours after treatment, the fruit were wounded with the sterile dissecting needles, which made two uniform holes (4 mm deep and 2 mm wide) at the two opposite equatorial sides of each fruit. Then fruit were inoculated with 15 μL of *P. expansum* at 1×10^4 spores mL^{-1} in each wound. All infected fruit were sealed in polyethylene bags (< 0.04 mm), and stored at 25 °C, 80–90% relative humidity (RH) for disease development. Lesion diameter and decay index of peach fruit were recorded on the 8th day after inoculation, using the method described below. Each treatment included 3 replicates of 10 fruits each. The experiment was performed twice.

Analysis of quality parameters of 5 fruits in each triplicate was performed on the 8th day of storage. Firmness, soluble solids contents, pH values, and titratable acidity in peach fruit were determined with the methods as follow:

Firmness of pulp was referred to Xi et al. (2017), using a force gauge with a 0.1–2 cm diameter flat probe (Zhejiang Tuopu Instrument Co., Ltd.). Soluble solids contents (SSC) were measured as the method described by Zhu et al. (2015), with a digital refractometer (PAL-1, Atago, Tokyo, Japan). pH values were detected by a pH -meter (PHS-3C, Shanghai, China). Titratable acidity (TA) was determined as the method reported early by Zhu et al. (2015) with some modifications. 5 g of fresh peach diluted with 5 mL of distilled water was titrated with 0.05 N NaOH to pH 8.2. Result was expressed as percentage of malic acid.

2.3. Effect of inoculation time after CGA treatment on controlling blue mold rot caused by P. expansum in peach fruit

Peach fruit were infiltrated with sterile distilled water (as control) or CGA at 100 mg L^{-1} under vacuum (−0.02 MPa) for 2 min, then air-dried at room temperature and inoculated with 15 μL of *P. expansum* at 1×10^4 spores mL^{-1} after 0, 12, 24, 36, or 48 h. All infected fruit were sealed in polyethylene bags (< 0.04 mm), and stored at 25 °C, 80–90% RH for disease development. Lesion diameter and decay index of peach fruit were recorded on the 8th day after inoculation, using the method described below. Each treatment included 3 replicates of 10 fruits each. The experiment was performed twice.

2.4. Effect of CGA on growth and spore germination of P. expansum in vitro

The effect of CGA on spore germination of *P. expansum* in vitro was assayed according to Yu et al. (2014) with some modifications. A CGA solution and 5 mL of potato dextrose broth (PDB) were mixed to obtain final concentrations of CGA at 0 (as control), 25, 50, 100, and 150 mg L^{-1} in sterile glass tubes. After adding a *P. expansum* suspension (1×10^6 spores mL^{-1}) to each, the tubes were placed in a rotary shaker in a water bath (200 rpm, 28 °C) and incubated for 24 h. The germination rate of approximately 200 spores was determined for each treatment replicated three times. The experiment was performed twice.

The effect of CGA on mycelial growth of *P. expansum* in vitro was performed according to Yao and Tian (2005) with some modifications. Different concentrations of CGA (0, 25, 50, 100, and 150 mg L^{-1}) were prepared by mixing with 20 mL of molten PDA in a petri dish (90 mm in diameter). After the PDA solidified, a sterile blank paper disk (1 cm in diameter) was placed at the center of each petri plate. Then a 50 μL of spore suspension (1×10^6 spores mL^{-1}) was placed on each paper disk with a sterile pipette. Colony diameter of *P. expansum* mycelial growth was determined after 3 days of incubation at 28 °C. There were three replicate groups for each treatment. The experiment was performed twice.

2.5. Experimental design for CGA treatment and evaluation of disease severity

Peach fruit were randomly divided into two groups containing 360 fruits each. This two groups were infiltrated with sterile distilled water and CGA at 100 mg L^{-1} under vacuum (−0.02 MPa) for 2 min, respectively, and air-dried. Each group was then divided into two subgroups containing 180 fruits each. Fruit in one of the subgroups in each main group were inoculated with 15 μL of *P. expansum* at 1×10^4 spores mL^{-1} 24 h after treatment according to the method described above. The remaining subgroup in each main group was not subjected to artificial inoculation. Therefore, the four groups in this work displayed as follows: 1) control, fruit treated only with sterile distilled water; 2) CGA, fruit treated only with CGA solution; 3) *P.* exp, fruit treated with sterile distilled water followed by inoculation with *P. expansum*; 4) CGA + *P.* exp, fruit treated with CGA at 100 mg L^{-1} followed by inoculation with *P. expansum*. All the fruit were sealed in polyethylene bags (< 0.04 mm) was stored at 25 °C, 80–90% RH. Flesh tissue of 6 fruits in each replicate was sampled and frozen immediately with liquid nitrogen at a certain interval time during storage. There were three replicate groups of 60 fruits in each treatment. The experiment was performed at least twice. Samples collected were stored at −80 °C for subsequent measurement of biochemical parameters.

Decay index (DI) of fruit was assessed according to Cao, Yan, Zhao,