Food Chemistry 228 (2017) 497–505

Contents lists available at ScienceDirect

Food Chemistry

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

DNP and ATP induced alteration in disease development of *Phomopsis longanae* Chi-inoculated longan fruit by acting on energy status and reactive oxygen species production-scavenging system

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ARTICLE INFO

Article history:
Received 20 October 2016
Received in revised form 8 February 2017
Accepted 10 February 2017
Available online 11 February 2017

Keywords:
Longan (*Dimocarpus longan* Lour.) fruit
Pericarp browning
Disease development
Energy status
Active oxygen metabolism
Phomopsis longanae Chi
2,4-Dinitrophenol (DNP)
Adenosine triphosphate (ATP)

ABSTRACT

As compared with *P. longanae*-inoculated longans, DNP treatment for *P. longanae*-inoculated longans exhibited higher fruit disease index and pericarp browning index, lower ATP amount and energy charge level, lower activities of SOD, CAT and APX, lower amounts of AsA and GSH, lower levels of DPPH radical scavenging activity and reducing power, higher $O_2^{\cdot-}$ generating rate and MDA amount. However, supply of ATP for *P. longanae*-inoculated longans showed the contrary effects. These results gave convincing evidence that DNP treatment for accelerating pericarp browning and disease development of harvested longans caused by *P. longanae* was due to decreases of energy production and ROS scavenging capacity, and increases of $O_2^{\cdot-}$ accumulation and membrane lipid peroxidation. Whereas, supply of ATP for retarding pericarp browning and disease development of harvested longans caused by *P. longanae* was due to increases of energy production and ROS scavenging capacity, and reductions of $O_2^{\cdot-}$ accumulation and membrane lipid peroxidation.

1. Introduction

Longan (*Dimocarpus longan* Lour.) fruit are one of the most commercially major tropical and subtropical fruits cultivated widely in many countries (Lin, Chen, Chen, & Hong, 2001; Lin et al., 2013, 2014; Lin, Lin, Lin, et al., 2016; Lin, Lin, Lin, Shi, et al., 2017). Since longan fruits harvest at high temperature season, the harvested longans have a short shelf-life featured by pericarp browning, fruit decay and disease development (Duan et al., 2007, 2011; Holcroft, Lin, & Ketsa, 2005; Lin et al., 2001). Pathogen invasion is one of the major factors resulting in the occurrences of pericarp browning and fruit disease in harvested longans (Holcroft et al., 2005; Lin et al., 2001). In addition, *Phomopsis longanae* Chi (*P. longanae*) is the primary pathogenic fungus of harvested longans (Chen et al., 2014). Although pre-harvest fruit bagging which controlled *P. longanae* infection for prolonging the shelf-life of harvested longans has certain effect, there are still absences of effective ways to inhibit pathogen infection (Chen et al., 2014; Lin, Weng, Zhang, Liang, & Lin, 2006). In order to develop more effective means to inhibit *P. longanae* infection for extending the shelf-life of harvested longans, it is essential for understanding metabolism of *P. longanae*-induced developments of pericarp browning and fruit disease in harvested longans.

Previous studies have shown that the loss of disease resistance and the developments of browning and disease of harvested horticultural crops might result from energy deficiency (Jiang et al., 2007; Jin, Zhu, Wang, Shan, & Zheng, 2013; Li, Limwachiranon, Li, Du, & Luo, 2016; Li, Zheng, Liu, & Zhu, 2014; Lin, Lin, Lin, Ritenour, et al., 2017; Liu et al., 2007; Saquet, Streif, & Banerth, 2003; Saquet, Streif, & Bangerth, 2000; Veltman, Lentheric, Van der Plas, & Peppelenbos, 2003; Zhou et al., 2014) and metabolic disorder of reactive oxygen species (ROS) (Lin, Xi, & Chen, 2005; Lin et al., 2014; Tian, Qin, & Li, 2013; Yi et al., 2010). Furthermore, adenosine triphosphate (ATP) is essential for the synthesis of ROS scavenging enzymes including superoxidase dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), and non-enzyme scavengers like ascorbic acid (AsA) and glutathione (GSH) (Tian et al., 2013; Yi et al., 2008, 2010). The levels of energy status also play a vital role in ROS production-scavenging system during the loss of disease resistance and the occurrences of browning and disease in harvested horticultural crops (Yi et al., 2008, 2010).

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http://dx.doi.org/10.1016/j.foodchem.2017.02.045
0308-8146/

Previous studies showed that adequate and valid energy might account for better maintenance of disease resistance and lower deterioration like browning of harvested horticultural crops (Chen et al., 2015; Jiang et al., 2007; Song et al., 2006; Wang et al., 2013; Yi et al., 2008). As storage time progressed, a decrease of ATP content and an increase of pericarp browning index in harvested longans were observed in our previous works (Chen et al., 2009). Moreover, hydrogen peroxide (H_2O_2) (Lin, Lin, Lin, Chen, et al., 2016) or 2,4-dinitrophenol (DNP, a respiratory uncoupling agent) (Chen et al., 2009) could promote browning development in pericarp of harvested longans by reducing the levels of energy charge and ATP content, and lowering ROS scavenging capacity. In addition, increases in pericarp browning index, fruit disease incidence, pericarp cellular membrane permeability and adenosine monophosphate (AMP) content, as well as decreases in amounts of ATP, adenosine diphosphate (ADP) and energy charge were observed in *P. longanae*-infected longans during storage (Chen et al., 2014). The results indicated that the shortage of energy caused by *P. longanae* infection might lead to an accelerating senescence and a reducing resistance to pathogen, which would stimulate the developments of pericarp browning and fruit disease in harvested longans (Chen et al., 2014). However, little literature is available on the roles of energy status and ROS production-scavenging system in the developments of pericarp browning and fruit disease in harvested longans induced by *P. longanae*. In this effort, the effects of DNP or ATP on the changes in energy status and ROS production-scavenging system of *P. longanae*-inoculated longan fruit and its relationship to pericarp browning and disease development were studied. The purposes of this investigation were to understand the roles of energy status and ROS production-scavenging system in the developments of pericarp browning and fruit disease in harvested longans induced by *P. longanae* infection.

2. Materials and methods

2.1. Materials and treatments

2,4-Dinitrophenol (DNP) (CAS 51-28-5, analytical grade), adenosine 5′-triphosphate disodium salt (one kind of ATP, CAS 987-65-5, analytical grade) were purchased from Sigma-Aldrich Corp, St. Louis, Missouri, USA.

P. longanae was separated from infected 'Fuyan' longan fruit and preserved in our laboratory. Spores of *P. longanae* was cultured on Oats bran medium at 28 °C for 15 days, the spore suspension of 10^5 spores mL^{-1} was prepared according to our previous study (Chen et al., 2014).

The fruits of mature longan cv. 'Fuyan' were picked from longan orchard at Quanzhou Xinyuanxing Agriculture Development Co., Ltd., Hui'an, China. The tested longan fruits were selected according to our previous study (Chen et al., 2015).

In our preliminary experiment, longan fruits were infiltrated with 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mM DNP under vacuum (80 kPa for 5 min). After air-drying for 60 min, the DNP-treated fruits were inoculated by *P. longanae* (10^5 spore mL^{-1}) and then stored at (28 ± 1) °C. The results showed that, as compared with the control fruits, there was no significant difference of the disease index in 0.1 or 0.2 mM DNP-treated *P. longanae*-inoculated longans, additionally, there was significant difference of the disease index in 0.3, 0.4 or 0.5 mM DNP-treated *P. longanae*-inoculated longans. However, 0.4 or 0.5 mM DNP-treated *longanae*-inoculated longans displayed rapid disease development, which was inconvenient for evaluating disease index and the related properties. Therefore, 0.3 mM DNP solution was used in this work. The selection of suitable ATP solution (0.8 mM) in this work was according to the results as described in our published paper (Chen et al., 2015).

The selected 150 longans were used for measuring the attributes of longan fruit at harvest day (day 0), and another selected 9000 longans were randomly divided into 9 lots (1000 fruit per lot) for control, DNP or ATP treatment. 3 lots were used for control, the other 3 lots for DNP treatment, and the another 3 lots for ATP treatment were assigned. Three replicates of 1000 longans (1 lot) for a total of 3000 longans (3 lots) were surface-sterilized with 0.5% (v/v) NaClO solution for ten seconds and washed with sterile distilled water, then infiltrated with sterile distilled water (control), 0.3 mM DNP solution or 0.8 mM ATP solution under vacuum (80 kPa for 5 min), respectively. The vacuum-infiltrated fruit were then air-dried for 60 min before pathogen inoculation at the harvest day.

2.2. Pathogen inoculation

The control longans, the DNP-treated longans and the ATP-treated longans as described above were inoculated by dipping into the *P. longanae* spore suspension of 10^5 spore mL^{-1} for 5 min, respectively. After inoculation, longans were packaged separately in sealed polyethylene film (0.015 mm thick) bags (50 longans per bag, 20 bags of each replicate and treatment), and stored at (28 ± 1) °C and 90% relative humidity. During storage, fruit of 3 bags (total 150 longans) from each replicate and treatment were sampled at random for evaluating pericarp browning and fruit disease, and for determining physiological and biochemical indices. Determinations of all indices were performed in triplicate.

2.3. Evaluation of pericarp browning and fruit disease

The methods of our previous studies (Lin et al., 2005, 2013; Lin, Lin, Chen, et al., 2016) were used to evaluate longan pericarp browning, the calculation of pericarp browning index was conducted according to the methods of Lin et al. (2005, 2014).

Longan fruit disease was assessed by the methods of our previous studies (Chen et al., 2014, 2015). The calculation of fruit disease index was conducted according to the method of Chen et al. (2014).

2.4. Determination of ATP, ADP and AMP content and energy charge

The extraction and determination of ATP, ADP and AMP content were conducted according to the methods of previous studies (Chen et al., 2014; Yang et al., 2009; Yi et al., 2008). The method of Chen et al. (2014) was used to calculate the level of energy charge.

2.5. Measurement of superoxide anion ($O_2^{\cdot-}$) generating rate and malondialdehyde (MDA) content

The methods of our previous studies were used to determine $O_2^{\cdot-}$ generating rate and MDA content (Lin et al., 2014, 2015). They were expressed as the unit of nmol g^{-1} min^{-1} and mmol g^{-1}, respectively.

2.6. Assay of SOD, CAT and APX activity

The methods of our previous studies were used to determine activities of SOD, CAT and APX, and protein content (Lin et al., 2005, 2014, 2015). The unit of U mg^{-1} protein was employed to express SOD, CAT and APX activity.

2.7. Determination of amounts of ascorbic acid (AsA) and glutathione (GSH)

The methods of Lin et al. (2005, 2014, 2015) were employed to determine amounts of AsA and GSH. The unit of g kg^{-1} was used to express the amounts of AsA and GSH.