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Effect of acibenzolar-*S*-methyl on energy metabolism and blue mould of Nanguo pear fruit

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ABSTRACT

Cellular energy is closely related to fruit ripening, senescence, physiological disorder and disease resistance after harvest. Nanguo pear fruit were immersed in 100 mg L^{-1} Acibenzolar-*S*-methyl (ASM) for 10 min, then air-dried at ambient conditions and stored for 12 d at 20 °C to investigate the effect of ASM on the activity of enzymes involved in energy metabolism. The effect of ASM on lesion development of the pear fruit inoculated with *Penicillium expansum* was also investigated in this study. Same treament with distilled water was used as control. The results demonstrated that ASM dipping treatment significantly decreased lesion diameter on the pear fruit inoculated with *P. expansum*. ASM dipping treatment enhanced ATP content, energy charge, the activity of H^+-ATPase, Ca^{2+}-ATPase, cytochrome C oxidase and succinic dehydrogenase in pear fruit. These results suggest that postharvest ASM treatment induced disease resistance in Nanguo pear fruit through mediating energy metabolism.

1. Introduction

Nanguo pear belongs to *Pyrus ussuriensis* Maxim and is mainly cultivated at the western of Liaoning province in China. The fruit is welcomed by consumers due to its bright color, good taste and pleasant flavor. However, harvested Nanguo pear fruit deteriorate rapidly due to water loss, core browning, flesh browning, ripening (Cheng et al., 2015; Li et al., 2014) and disease development caused by fungus, among which *Penicillium expansum* is the most important (Errampalli, 2014). Many effective chemical fungicides have been prohibited to application due to the problems related to the development of pathogen resistance and the potential adverse effects on the environment and human health (Romanazzi et al., 2016). Inducing resistance by physical, chemical and biological agents, however, is becoming a new potential strategy to both manage postharvest decay and keep the quality of fruit (Youssef et al., 2014; Romanazzi et al., 2016).

Acibenzolar-*S*-methyl (ASM) has been reported as an effective elicitor to inducing disease resistance in many fruit and vegetables including mango (Zhu et al., 2008), orange (Moscoso-Ramírez and Palou, 2013), banana (Tang et al., 2010), muskmelon (Ge et al., 2008; Ren et al., 2012), apple (Sklodowska et al., 2010), pear (Cao et al., 2005), and strawberry (Cao et al., 2010, 2011). Moreover, application of ASM could maintain postharvest quality of muskmelon (Ren et al., 2013) and peach fruit (Liu et al., 2005). Studies have shown that mechanisms involved in inducing resistance by ASM including the accumulation of reactive oxygen species, activating phenylpropanoid pathway, accumulation of pathogenesis-related proteins, energy metabolism and genes expression related to these defense responses (Liu et al., 2014; Ge et al., 2015; Li et al., 2015b).

Energy metabolism plays key roles in fruit and vegetables ripening, senescence and disease resistance after harvest, which are responsible for maintaining a higher quality in fruit and vegetables during storage (Huang et al., 2014). Studies on kiwifruit, peach, loquat, litchi, pear, and banana showed that the senescence process and disease resistance were closely related to the cellular energy status (Cao et al., 2014; Chen et al., 2014; Huang et al., 2014; Jin et al., 2013; Wang et al., 2013; Yi et al., 2010). Pre-harvest or postharvest application of methyl jasmonate (MeJA) (Cao et al., 2014), oxalic acid (Jin et al., 2014), nitric oxide (Li et al., 2015a), 1-MCP (Cheng et al., 2015), ATP (Yi et al., 2009; Yi et al., 2010) can maintain high contents of ATP in fruit and vegetables. However, little information was reported on the effect of ASM dipping treatment on the activities of energy metabolism-related enzymes and disease resistance of Nanguo pear fruit during storage.

The aim of current study is to evaluate the effects of ASM treatment after harvest on energy metabolism-related enzymes activities, energy charge, and lesion development in Nanguo pear fruit during storage at 20 °C.

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2. Materials and methods

2.1. Plant material and chemical

Pear fruit (*Pyrus ussuriensis* cv. Nanguo) were sampled at a commercial mature stage (70–80%) from the orchard in Jinzhou, Liaoning Province. Fruit were selected based on color, size and absence of diseases or physical injuries, then washed, disinfected with sodium hypochlorite (2%, v/v) for 1 min and air-dried at ambient conditions. Acibenzolar-*S*-methyl (ASM, ai. 50%) was kindly provided by Syngenta Company.

2.2. Treatment

Disinfected fruit were immersed in 100 mg L^{-1} ASM (containing 0.05% Tween-20) solution for 10 min. Control fruit were similarly treated with distilled water containing 0.05% Tween-20. All treated fruit were air-dried at ambient conditions, then put into ringent ziplock bag and stored at 20 °C for following experiments. Three replicates were made, each replicate containing 50 fruit, and the whole experiment was performed three times.

2.3. Sample collection

Samples were collected according to the method of Ge et al. (2008) and Ren et al. (2012). Tissues (3 g) were cut from 0.2 to 0.8 cm under the epidermis around the equator of pear fruit at 0, 2, 4, 6, 8, 10, 12 d after treatment. For mitochondria extraction, 25 g of sample were detached. Each sample was wrapped in aluminum-foil paper, immediately frozen in liquid nitrogen and preserved in refrigerator at −80 °C for following assay.

2.4. Pathogen preparation and inoculation

P. expansum was originally isolated from infected pear fruit and cultured on potato dextrose agar (PDA) at 25 °C. Spore suspensions were prepared by flooding the culture plates with 4–5 mL of sterile distilled water and filtered with double-layer cloth. The inoculum was adjusted to 1×10^6 spore mL^{-1} and confirmed by a haemocytometer. Inoculation was carried out in 24 h. The fruit were surface-sterilized with 75% ethanol, and then four wounds were made with a sterilized needle (3 mm deep by 2 mm wide) around the equator of each fruit. Twenty microlitre of the spore suspension was injected into each wound. After drying under air condition for approximately 1 h, the fruit was put in ringent ziplock bag and incubated at 20 °C (RH 60–70%). The lesion diameter was recorded 3 d after inoculation. ASM treatment and control contained three replicates with 30 fruit for each replicate, and the entire experiment was performed three times.

2.5. Mitochondria extraction

Crude mitochondria were isolated from pear fruit according to the method of Jin et al. (2014) with minor modifications. Briefly, frozen flesh tissues (25 g) was homogenized in 50 mL of Tris-HCl buffer (pH 7.6, 0.05 M) containing 0.01 M ethylene diamine tetraacetic acid (EDTA), 0.25 M sucrose, 0.3 M mannitol, 5 g L^{-1} polyvinylpyrrolidone (PVP) and 1 g L^{-1} bovine serum albumin (BSA) at 4 °C for 10 min. The following steps were same as Jin et al. (2014).

2.6. Determining of energy metabolism-related enzymes activities

The activities of H^+-ATPase, Ca^{2+}-ATPase, cytochrome C oxidase (CCO) and succinate dehydrogenase (SDH) were measured following the method of Zhou et al. (2014). H^+-ATPase and Ca^{2+}-ATPase activities were expressed as U/mg protein, where U was defined as the release of 1 μmol of phosphorus at 660 nm per second. The activity of CCO was expressed as U mg^{-1} protein, where $U = 0.01\Delta A_{510}/s$. The activity of SDH was expressed as U mg^{-1} protein, where $U = 0.01\Delta A_{600}/s$.

The content of protein in enzyme extracts was measured by the method of Bradford (1976) using bovine serum albumin as a standard.

2.7. Assay the content of ATP, ADP, AMP and energy charge

The extraction and assay of ATP, ADP and AMP contents were following the method of Jin et al. (2013) with slight modifications. Briefly, frozen sample (3 g) was homogenized in 5 mL of perchlori acid (0.6 M) at 4 °C for 3 min. The homogenates were centrifuged for 20 min at 13,000 × *g* at 4 °C. Three milliliter of the supernatant was taken and adjusted the final pH to 6.5-6.8 with 100 mM potassium hydroxide solution, then diluted with double-distilled water to 5 mL. The content of ATP, ADP and AMP was determined using an HPLC (Agilent 1100) equipped with a reverse-phase C_{18} column (4.6 mm × 250 mm) and an ultraviolet detector at 254 nm. The separation was achieved using a linear gradient program with 75–100% phase A (0.03 M K_2HPO_4 and 20 mM KH_2PO_4) and 0–25% phase B (pure acetonitrile) for 9 min with the flow rate of 1.0 mL s^{-1}, and the injection volume was 10 μL. The content of ATP, ADP, and AMP in the sample was determined using the master standard and expressed as g kg^{-1}. Energy charge = [ATP + ADP]/[ATP + ADP + AMP].

2.8. Data analysis

The data were analyzed by one-way ANOVA using SPSS 22.0 software (SPSS inc., Chicago USA). The means were compared using Fisher's least significant differences (LSD, $P < 0.05$).

3. Results

3.1. Effects of ASM treatment on lesion development of pear fruit

Lesion diameter developed with storage time, postharvest ASM dipping significantly decreased ($P < 0.05$) the lesion diameter of blue mould in pear fruit caused by *P. expansum* compared to the control (Fig. 1).

3.2. The activity of H^+-ATPase, Ca^{2+}-ATPase, CCO and SDH

The activity of H^+-ATPase and Ca^{2+}-ATPase declined gradually during assay period in both ASM-treated and control fruit (Fig. 2A and B). ASM dipping significantly enhanced ($P < 0.05$) the activity of H^+-

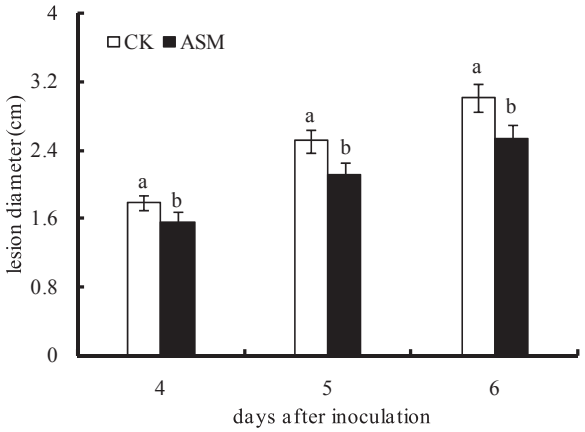

Fig. 1. Effects of postharvest ASM treatment on lesion diameter on the pear fruit inoculated with *P. expansum*. Bars represent standard error of the means. Values with a common letter do not differ significantly between treatments according to LSD at $P < 0.05$.