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6-Benzylaminopurine improves the quality of harvested litchi fruit

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ABSTRACT

6-Benzylaminopurine (BAP), a synthetic cytokinin, can elicit plant growth and development by stimulating cell division. In this study, the effects of BAP on decay and pericarp browning of harvested litchi fruit in relation to phenolics and ROS metabolism were investigated. Application of BAP significantly inhibited decay incidence of harvested litchi, associated with a direct inhibition on *Peronophythora litchii,* the major pathogenic fungi. In addition, BAP-treated fruit showed significantly lower pericarp browning, accompanied by reduced PPO activity, increased PAL activity and higher contents of anthocyanin and total phenolic compounds. Moreover, BAP reduced H_2O_2 accumulation and lipid peroxidation, which may account for browning inhibition to an extent. Furthermore, higher activities of SOD, CAT and APX and DPPH radical scavenging capacity in BAP-treated fruit possibly benefited reducing ROS accumulation and lipid peroxidation. Overall, application of BAP showed great potential to control decay and browning and extend shelf life of harvested litchi.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is an important commercial fruit in some subtropical countries, including China, India, Australia, Thailand, and Israel. The fruit is characterized by semi-translucent white aril and attractive red skin. However, litchi fruit is highly perishable after harvest. The major problem for the fruit is decay development and pericarp browning, leading to short shelf life under ambient condition (Jiang et al., 2004). It is estimated that loss of harvested litchi is up to 20% of total output due to poor postharvest technology (Zhang et al., 2004a,b).

The mechanism underlying pericarp browning of harvested litchi fruit is complex. Previous studies have showed that pericarp browning of litchi fruit is mainly attributed to enzymatic reaction, anthocyanin degradation and microbial infection (Ali et al., 2016). Enzymatic oxidation of phenolics by phenoloxidase (PPO) and peroxidase (POD) is considered as one of the major causes resulting in pericarp browning. Anthocyanin degradation due to enhanced pH and over-production of reactive oxygen species (ROS) is also an important factor. In addition, litchi after harvest is susceptible to infection of pathogens, among which *Phytophthora litchii* is one of the most common pathogenic fungi (Zhou et al., 2016). The invasion of *P. litchii* may cause metabolism disorder and loss of membrane integrity, leading to pericarp browning of litchi fruit (Gong et al., 2016; Liu et al., 2006). Therefore, delaying physiological activity and inhibiting pathogen development may alleviate pericarp browning and improve overall quality of litchi fruit after harvest.

Postharvest technologies, including sulfur fumigation, acid dips, fungicides, heat treatment, packaging and modified atmosphere packaging, have been developed to reduce pericarp browning and decay development (Jiang et al., 2006). Of these technologies, sulfur fumigation is considered the most effective and practical treatment in controlling pericarp browning of litchi fruit (Ray et al., 2005). Sulfur dioxide can inhibit enzymatic browning by inactivating PPO activity (Holcroft and Mitcham, 1996). Considering potential safety issues, it is necessary to develop effective commercially alternative approaches instead of sulfur fumigation for postharvest litchi management.

Cytokinins are a class of phytohormones that can promote cell division (Hwang et al., 2012), playing a role in regulating plant growth, development and differentiation (Gan and Amasino, 1996; Markovich et al., 2017). 6-Benzylaminopurine (BAP) is the first synthetic cytokinin. Previous studies have shown that application of BAP effectively delay senescence and improve quality of chlorophyll-containing vegetables in relation to inhibition of chlorophyll degradation (Massolo et al., 2014; Wei and Ye, 2011; Xu et al., 2013). Moreover, a recent study has shown that BAP reduces brown rot caused by *Monilinia fructicola* in peach fruit during storage (Zhang et al., 2015). Despite

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beneficial effect, limited information on the role of BAP in regulating senescence of harvested litchi fruit is available.

The objective of the present study was to investigate the effects of BAP on the enzymatic browning and oxidative stress of harvested litchi fruit. The inhibitory effect of BAP on *P. litchii* was also investigated. The results will help to develop a relatively novel non-toxic approach to maintain quality and improve economic value of harvested litchi fruit.

2. Materials and methods

2.1. Fruit material and treatments

Litchi (*Litchi chinesis* Sonn) were harvested from a commercial orchard at Guangzhou, China, and immediately transported to the laboratory. Fruit with uniformity of shape, color and size were used. Preliminary investigations showed that, within a concentration range from 0.01 to 0.5 g L^{-1}, application of 0.1 g L^{-1} BAP was most effective in inhibiting pericarp browning of litchi fruit stored for 8 d at 25 °C. In this study, three hundred fruit were dipped for 10 min in.0.1 g L^{-1} BAP. Control fruit (300) were dipped in distilled water. Fruit were then air-dried for 1 h, packed with polyethylene bags (0.03 mm film-thickness; 4 holes that are 5 mm in diameter per bag; 20 fruit per bag), and stored at 25 °C. Each treatment had three replicates. During storage, the fruit were randomly sampled and analyze at 2-d intervals.

2.2. Assessment of pericarp browning and fruit decay

Pericarp browning was assessed by the method of Jiang (2000), expressed as browning index. Decay incidence was monitored by collecting 60 fruit (3 bags) and then recording the percentage showing fungal growth. When browning index was more than 3.0 or the fruit showed decay, the fruit was considered as unacceptable for market.

2.3. Effect of BAP on mycelial growth of P. litchii in vitro

An isolate of *P. litchii* was provided kindly by South China Agriculture University. *P. litchii* was cultured on PDA plates for 5–7 d. Fungal cakes with a 5-mm diameter were collected on the edge of *P. litchii* colony and then inoculated on PDA plates containing 0, 0.001, 0.01, 0.05 or 0.1 g L^{-1} BAP. BAP was dissolved in a minimum of ethanol. The same amount of ethanol was contained in the PDA plates, compared with the PDA plate with 0.1 g L^{-1} BAP. The 0.05 g L^{-1} prochloraz was used as a positive control. The colony diameter was measured 120 h after dark incubation at 25 °C. Inhibition rates (%) of mycelial growth were calculated as [(control colony diameter − treated colony diameter)/(control colony diameter − fungal cake diameter)] × 100. Each treatment had three replicates.

2.4. Contents of anthocyanin and total phenolic compounds

Anthocyanin contents in litchi pericarp were determined by the method of Zhang et al. (2004a,b), expressed as milligrams of cyanidin-3-glucoside equivalent on a fresh weight basis. The contents of total phenolic compounds in litchi pericarp were measured using Folin-Ciocalteu reaction (Singleton and Rossi, 1965), expressed as gallic acid equivalents in milligrams on a fresh weight basis.

2.5. Measurement of polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities

2.0 g of pericarp tissues were ground in liquid nitrogen and homogenized in 0.02 L of 0.05 M phosphate buffer (pH 7.0) and 0.5 g of polyvinylpyrrolidone (insoluble) at 4 °C. The homogenate was centrifuged at 19 000*g* for 20 min at 4 °C and the supernatant was used as the crude enzyme extract for the analysis of PPO activity according to the method described by Jiang (2000). 4.0 g of pericarp tissues were ground in liquid nitrogen and homogenized in 0.02 L of 0.1 M Na-borate buffer (pH 8.0) containing 5 mM β-mercaptoethanol and 2 mM EDTA and 0.25 g of polyvinylpyrrolidone (insoluble), at 4 °C. After centrifugation for 20 min at 19 000*g* and 4 °C, the supernatant was used for PAL activity assay according to the method described by Jiang and Joyce (2003). The activities of PPO and PAL were expressed on a protein basis. Protein contents were determined by Bradford (1976), using bovine serum albumin as the standard.

2.6. Respiratory rate

The respiration rate of whole fruit was determined by infrared gas analyzer. Fifteen fruit were sealed in a 2.5 L plastic box at 25 °C. Increases in CO_2 concentration in the box were monitored for 5 min using an infrared gas analyzer (Li-6262 CO_2/H_2O analyzer) by passing air stream. Respiration rates were released as increased CO_2 per second on a fresh weight basis.

2.7. H_2O_2 and malondialdehyde (MDA) contents

H_2O_2 content of litchi pericarp was determined using a Hydrogen Peroxide Assay kit (Jianchen Biotech Company, Nanjing, China) in accordance with the manufacturer's instructions. MDA content of litchi pericarp was measured using thiobarbituric acid method (Duan et al., 2011). H_2O_2 and MDA contents were expressed on a fresh weight basis.

2.8. Antioxidant enzymes activities

2.0 g of pericarp tissues were ground in liquid nitrogen and homogenized in 0.01 L of 0.2 M phosphate buffer, pH 7.0, containing 0.2 g PVP and 0.1 mM EDTA at 4 °C. After centrifugation at 20 000*g* and 4 °C for 15 min, the supernatant was used as the crude enzyme for assays of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities. Activities of SOD, CAT and APX were determined according to the methods described by You et al. (2012). The activities of SOD, CAT and APX were expressed on a protein basis.

2.9. Diphenol-1-picrylhydrazyl radical (DPPH) radical scavenging activity

3.0 of pericarp tissues were ground in liquid nitrogen and extracted for 2 h in.0.05 L methanol containing 0.5% (w/v) sodium metabisulphite. After filtration, the filtrate was used for evaluating DPPH radical scavenging activity by the method of Duan et al. (2007).

2.10. Statistical analysis

The data were analyzed with SPSS 13.0 statistical software (SPSS Inc., Chicago, USA) by an ANOVA test, followed by a least significant difference (LSD) test to compare significant effects at 5% level.

3. Results

3.1. Fruit browning and decay

Pericarp browning and decay development are the major problems of harvested litchi fruit. As shown in Fig. 1A, severe browning and decay symptoms developed after 8 d of storage at 25 °C. However, much less pericarp browning and fruit decay were observed in BAP-treated fruit 8 d after storage at 25 °C. Fig. 1B showed the effect of BAP treatment on pericarp browning of harvested litchi fruit during 8 d of storage. The browning index of litchi fruit rapidly increased with increased storage time, indicating that the fruit turned brown gradually. Fruit treated with BAP had a lower pericarp browning index, compared with the control fruit. After 8 d of storage, the browning indices were 3.8 and 2.1 for control fruit and BAP-treated fruit, respectively (Fig. 1A). No obvious decay symptom was observed within the first 4 d