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Effects of hot water treatment on anthracnose disease in papaya fruit and its possible mechanism

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ABSTRACT

Harvested papaya fruit are perishable due to rapid ripening and softening and susceptibility to biotic or abiotic stresses. Hot water treatment (HWT) can preserve fruit quality by reducing decay. The present study investigated effects of HWT on controlling fungal pathogens of papaya fruit and the possible mechanism by which HWT induced disease resistance. HWT (54 °C, 4 min) of papaya fruit had a pronounced effect on reducing the carrier rate of *Colletotrichum gloeosporioides* (*C. gloeosporioides*) in fruit peel, significantly inhibited the incidence of anthracnose and stem-end rot, effectively delayed fruit softening, but slightly promoted the rate of fruit coloring. HWT reduced the anthracnose index and fruit ripeness to a certain extent and induced changes in the wax arrangement on the surface of treated fruit, causing the wax to melt. The cracks and most stomata appeared to be partially or completely plugged by the melted wax, thereby providing a mechanical barrier against wound pathogens. HWT induced the expression of *CpPGIP* and promptly induced the expression of *CpNPR1*, and then regulated the expression of the *CpPR1* gene, which may enhance the resistance of the fruit to anthracnose disease and reduce the decay rate. Together, these results confirm that HWT could reduce disease incidence and induce resistance, and thus maintain postharvest quality during storage and prolong the shelf-life of papaya fruit.

1. Introduction

Papaya (*Carica papaya* L.) is a climacteric fruit and very perishable after harvesting, with rapid ripening and softening, and susceptibility to biotic or abiotic stresses (Kader, 2002). An import factor that may affect papaya postharvest quality and limit the extension of storage life is the occurrence of rots. Anthracnose is one of the most important postharvest diseases in papaya (Cia et al., 2007), as well as other fruit (Shi et al., 2011). Postharvest diseases, especially anthracnose, can infect young papaya fruit and remain latent during fruit growth in the field Thus it is hard to prevent disease development, and this may significantly reduce quality and commercial value of the fruit during storage and transport. Anthracnose is usually controlled by application of postharvest fungicides such as prochloraz or propiconazole. However, development of fungicide resistance, and public concern over the potential impact of fungicides on human health and the environment have created interest in seeking new alternative strategies for disease management (Gamagae et al., 2004; Droby et al., 2009). Extensive research is imperative to develop advanced postharvest treatments in environmentally friendly ways to maintain high commercial quality during the storage and marketing period. Different postharvest diseases of various fruit have been effectively controlled using modified atmosphere packaging (Karabulut and Baykal, 2004), sodium chloride (Malakou and Nanos, 2005), biocontrol (Shi et al., 2010), antagonistic microorganisms (Calvo et al., 2010), salicylic acid (Chan et al., 2008), borate application (Shi et al., 2011, 2012), etc.

Among various non-chemical approaches, hot water treatment (HWT) appears to be one of the most effective and promising methods, especially for organically grown crops, to control relatively high rates of postharvest decay in environmentally friendly ways (Mari et al., 2007; Jemric et al., 2011; Fruk et al., 2012; Liu et al., 2012). HWT has been known for a relatively long time as an effective and economic method for controlling plant pathogens (Rodov et al., 1995, 2000; Schirra and D'Hallewin, 1997; Hong et al., 2007; Liu et al., 2012), improving fruit resistance to chilling (Rodov et al., 1995; Schirra et al., 2004; Lu et al., 2010a; Rui et al., 2010), inhibiting ripening of many fruit and vegetables, and alleviating some physiological storage disorders (Fallik, 2004; Ciou et al., 2011; Jemric et al., 2011), thus, maintaining fruit quality and prolonging storage (Klein and Lurie, 1991; Malakou and Nanos, 2005; Fruk et al., 2012; Liu et al., 2012). There has therefore, been increasing interest in using postharvest heat treatments for fruit. Ripening of most climacteric fruit is characterized by softening of the flesh, enhanced color

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development, increased sugar/acid ratio, respiratory activity and ethylene production (Prasanna et al., 2007). Exposing fruit to high temperatures retards some of these processes while enhancing others. The physiological and biochemical changes in heat-treated fruit are more advanced in some ripening characteristics than non-treated fruit, thus maintaining the fruit quality for a longer period during storage and shelf-life.

Postharvest decay controlled by HWT, however, involves effects on both plant pathogen and plant host (Pavoncello et al., 2001; Liu et al., 2012). Several studies have reported that HWT had a pronounced effect on decreasing decay, reducing chilling injury and maintaining quality of fruit (Lu et al., 2010a; Fruk et al., 2012). However, improper temperature and duration of HWT can have a negative impact on fruit quality. On the other hand, other studies have reported that HWT could directly inhibit fungal germination and growth, or even kill the fungus (Jemric et al., 2011; Liu et al., 2012). With regard to the mechanisms of control of postharvest decay by HWT, several studies have indicated that HWT could induce a defense response of fruit and vegetables, sequentially preventing the pathogen spreading throughout the tissues (Ben-Yehoshua et al., 1995; Fallik et al., 1996b). With papaya fruit, while a few studies have applied heat treatments, including hot air and hot water, to control postharvest decay and for other commercial applications (Chan Jr et al., 1981; Nishijima et al., 1992; Lay-Yee et al., 1998; Kader, 2002; Diczbalis-Deedi et al., 2012), no systematic studies on effects of postharvest HWT on papaya fruit and the underlying molecular biology mechanisms have been reported.

Expansins are known to participate in several processes during plant growth and development, particularly where wall extension and cell expansion are required. They are also suggested to prepare cell walls for subsequent degradation by cell wall hydrolases during ripening, particularly in climacteric fruit. As a class of nonenzymatic cell wall proteins, expansins have been found to play an important role in cell wall loosening and extension (Cosgrove, 2000). Close relationships between expansins and postharvest fruit softening have been shown in a number of fruit (Wang et al., 2006; Yang et al., 2008). Fruit firmness is related to fruit quality and is negatively associated with the incidence of preharvest and postharvest rots (Barritt, 1980).

In the present study, a simple and effective HWT (54 °C, 4 min) for papaya fruit was screened and the physiological basis and molecular biology mechanism of HWT-induced systemic resistance studied. The overall objective of this work was to elucidate the physiological defense reactions and the possible molecular mechanisms of systemically acquired resistance.

2. Materials and methods

2.1. Plant materials and hot water treatment

Papaya fruit (*Carica papaya* cv. 'Sunrise') at physiological maturity indicated by a color break stage of 10–15% yellow, were harvested from a local commercial plantation near Guangzhou, South China, transported to the laboratory and sorted by size, shape and maturity. Uniform fruit free from visual symptoms of any disease or blemishes were randomly selected and first cleaned, and dipped in a 0.3% hypochloride solution for 10 min. Preliminary investigations revealed that treatment with 54 °C for 4 min was the most effective in controlling diseases and/or delaying the ripening of papaya fruit. The selected papaya fruit were dipped into hot water (54 °C) for 4 min, taken out and then allowed to air-dry at 25 °C. The control group was dipped into water at normal temperature water (25 °C) for 4 min. Every treatment was repeated three times and each treatment included 90 fruit. Thereafter, fruit were placed into unsealed plastic bags (0.02 mm thick) and stored at 25 °C. Samples were collected at 0, 12, 24 and 36 h and 2, 3, 4, 5, 6, 7, 8, 9 and 10 days after treatment. For all samples, peel around the equatorial fruit, about 2 mm thick, and 2 cm thick flesh, were collected, then frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Color index and disease index evaluation

Fruit peel color and coloring index were calculated as in our previous study (Zhu et al., 2012). Disease severity of anthracnose in terms of lesion diameter was classified into nine grades according to Lay-Yee et al. (1998): 0 = without lesion; 1 = 1–5 mm lesion diameter = <20; 2 = 6–15 mm lesion diameter; 3 = lesion proportion = <1/16; 4 = lesion proportion = <1/8; 5 = 1/8 = <lesion proportion = <1/4; 6 = 1/4 = <lesion proportion = <1/2; 7 = 1/2 = < lesion proportion = <2/3; 8 = decay completely. Use of this empirical scale made it possible to calculate a disease index (DI) showing the average disease severity as a proportion of the maximum disease severity. DI = [∑(disease grade × number of fruit with disease)/(total number of fruit × maximum disease grade)] × 100.

Periodic observations were made for incidence of stem-end rot, the severity of the stem-end rot disease being measured with the following scale: 0 = no disease; 1 = the proportion of the stem-end portion surface affected with disease <25%; 2 = the proportion of the stem-end portion surface affected with disease 25–50%; 3 = the proportion of the stem-end portion surface affected with disease 50–75%; 4 = the proportion of the stem-end portion surface affected with disease <75%; 5 = the disease spread to the other part of the fruit. DI = [∑(disease grade × number of fruit with disease)/(total number of fruit × maximum disease grade)] × 100.

2.3. The determination of carrier rate of Colletotrichum gloeosporioides in fruit peel

After being stored for 4 days, *Colletotrichum gloeosporioides* (*C. gloeosporioides*) was isolated from fruit peel both in the control fruit and the HWT-fruit. Fruit peel was cut into 2 mm × 2 mm portions, 1 mm thick, soaked in 75% alcohol for 10 s, and then soaked in 0.5% mercuric chloride for 2 min. The treated peel was then washed with sterile water 3 times and maintained on potato dextrose agar (PDA) at 28 °C for 3–4 days. The carrier rate of *C. gloeosporioides* was statistically analyzed according to the following formula:

$$\text{The carrier rate of } C.\ gloeosporioides = \left(\frac{\text{number of fruit with } C.\ gloeosporioides}{\text{total number of fruit}}\right) \times 100.$$

Thirty fruit were used for each treatment and each treatment was replicated three times.

During the *C. gloeosporioides* isolation process, the treated peels were maintained on potato dextrose agar (PDA) and used to isolate *C. gloeosporioides*. Whether or not the fungi were latent or active in the fruit peel, it could develop into colonies in the PDA after a few days of incubation.

2.4. Determination of pectin content

The method of Wang et al. (2008) was used to determine pectin content. A mixture of 5 g of sample powder and 30 mL of hot absolute ethanol was heated in a centrifuge tube for 10 min in a boiling water bath and centrifuged at 10,000 rpm for 10 min at 4 °C. The residues were dried for 24 h at 35 °C, and alcohol insoluble solids (AIS) were obtained. One milliliter of water was added drop-wise with stirring, for 35 min to a mixture of 5 mg of AIS and 2 mL of concentrated sulfuric acid, in a test-tube until the AIS were dissolved. The mixture was transferred to a 25 mL volumetric flask