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Enhanced resistance of jujube (*Zizyphus jujuba* Mill. cv. Dongzao) fruit against postharvest *Alternaria* rot by β-aminobutyric acid dipping

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ABSTRACT

Considerable losses of jujube (*Zizyphus jujuba* Mill. cv. Dongzao) fruit caused by *Alternaria* rot often occurred during storage. In order to evaluate effects of β-aminobutyric acid (BABA) on the infection on jujubes by *Alternaria alternata*, the fruit were dipped in BABA solutions and then inoculated with the pathogen. Results showed that BABA dipping at concentrations of 0.5, 1.0 and 2.0 g L^{-1} significantly ($P < 0.05$) reduced disease incidence and lesion area on the fruit inoculated with *A. alternata*, whereas BABA did not affect conidial germination and mycelial growth of the pathogen *in vitro*. In addition, BABA reduced natural infection and postharvest softening of jujubes during the storage at 0 °C and 85–95% relative humidity. Biochemical evaluations revealed that BABA enhanced activities of defence-related enzymes including peroxidase, phenylalanine ammonia-lyase and chitinase of the fruit. BABA altered antioxidant metabolism to trigger disease resistance by significantly ($P < 0.05$) decreasing catalase activity but increasing superoxide dismutase activity and ascorbic acid content in jujubes. These results suggested that the protective effects of BABA dipping on jujubes might be due to its ability on activating several highly coordinated defence-related responses of the fruit against infection, instead of its direct antifungal activity on the pathogen. These findings suggested that application of BABA would offer a promising approach for controlling postharvest disease and improving storage quality of horticultural products.

1. Introduction

Jujube (*Zizyphus jujuba* Mill.) fruit have been widely consumed as a traditional and functional food with numerous beneficial effects on human health-promoting in Asian countries (Gao et al., 2013; Zozio et al., 2014). However, fresh jujubes ripen easily and are highly susceptible to infection by pathogens, which greatly limits postharvest supply period and often leads to considerable losses of the fruit during storage. Previous studies have shown that black mould rot, caused by *Alternaria alternata* (Fr.) Keissl, is responsible for most of the postharvest losses of fresh jujubes, even when the fruit were stored at a low temperature (Tian et al., 2005; Cao et al., 2013a,b; Yan et al., 2011, 2012; Wang et al., 2009; Li et al., 2012). Recently, some alternatives to traditional synthetic fungicides have been developed to protect fresh jujubes against postharvest infections, including biological control using microorganisms (Tian et al., 2005; Wang et al., 2009; Cao et al., 2012), coating with chitosan (Wu et al., 2010; Wang et al., 2014), fumigation with 1-methylcyclopropene (Zozio et al., 2014; Zhang et al., 2012), application of Harpin protein (Li et al., 2012), oligochitosan (Yan et al., 2011, 2012) or salicylic acid (Cao et al., 2013a, b). Still, a search for new alternatives is becoming increasingly important for reducing postharvest decay of the fruit.

The non-protein amino acid, β-aminobutyric acid (BABA), has been shown to induce resistance in plant against various pathogenic infections by potentiating natural defence mechanism of plant tissue, though BABA itself does have direct antifungal activity on *in vitro* growth of pathogens (Fischer et al., 2009; Porat et al., 2003; Reuveni et al., 2003; Tavallali et al., 2008; Marcucci et al., 2010; Quaglia et al., 2011; Zhang et al., 2011). Interestingly, induction of disease resistance with BABA has emerged as one of the most promising alternatives to fungicides for control of postharvest decay of horticultural products (Reuveni et al., 2003; Tavallali et al., 2008; Quaglia et al., 2011). BABA can trigger the induced resistance in fruits and vegetables against various diseases, including green mould on grapefruit caused by *Penicillium digitatum* (Porat et al., 2003), blue mould on sweet orange caused by *Penicillium italicum*

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(Tavallali et al., 2008) and apple caused by *Penicillium expansum* (Quaglia et al., 2011; Zhang et al., 2011), moldy-core decay of apple caused by *A. alternata* (Reuveni et al., 2003), anthracnose on mango caused by *Colletotrichum gloeosporioides* (Zhang et al., 2013), dry rot of potato tuber caused by *Fusarium sulphureum* (Yin et al., 2010), storage decay of melons mainly caused by *Fusarium*, *Alternaria* and *Rhizopus* spp. (Bokshi et al., 2006), white mould on artichoke caused by *Sclerotinia sclerotiorum* (Marcucci et al., 2010), downy mildew rot of lettuce caused by *Bremia lactucae* (Cohen et al., 2010), neck rot of onion caused by *Botrytis* spp. (Polyakovskii et al., 2008). Thus, fungal decay and postharvest losses of horticultural products can be reduced by application of BABA.

In our previous studies, pre-harvest foliar sprays with BABA reduced incidence of postharvest diseases in the jujube fruit caused by *Monillinia fructicola* and *A. alternata* (Cao et al., 2013b). However, to date, no report is available regarding postharvest application of BABA on jujubes during storage.

Accordingly, the present work was undertaken to evaluate effects of postharvest application of BABA on *Alternaria* rot and natural infection of jujubes. Mechanisms involved in defence responses of the fruit as influenced by BABA were investigated. Additionally, *in vitro* tests were performed to estimate the direct antifungal activity of BABA on the growth of *A. alternata*.

2. Materials and methods

2.1. Fruit materials and treatments

Chinese winter jujube (*Z. jujuba* Mill. cv. Dongzao) fruit were harvested at green mature stage from a commercial orchard in Beijing, China. Jujubes were transported to the laboratory immediately after harvest and selected according to their uniformity of shape and size, and those with physical injuries or infections were discarded.

DL-β-aminobutyric acid (DL-3-amino-*n*-butyric acid, BABA) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and BABA solutions were prepared with distilled water containing 0.01% (vol/vol) Tween-20 as surfactant. Jujubes were dipped into 0 (as control), 0.5, 1.0 or 2.0 g L^{-1} BABA solution at ambient temperature (25 °C) for 20 min and then air-dried. A portion of the dipped fruit were incubated at 15 °C, 80–90% relative humidity (RH) for 24 h for inoculation and disease evaluation. Another portion of the dipped fruit were stored at 0 °C, 85–95% RH for estimation of storage quality and natural infection.

2.2. Preparation of the pathogen

A. alternata (Fr.) Keissler was isolated from the infected jujube fruit and maintained on potato dextrose agar (PDA) plates at 27 °C. Conidial suspension of the pathogen was prepared by washing a 12-day old culture dish with 5 mL of sterile distilled water containing 0.01% (vol/vol) Tween-20. The suspension was filtered through four layers of sterile cheesecloth to remove any adhering mycelia. Concentration of the conidial suspension of *A. alternata* was adjusted to 2×10^5 conidia mL^{-1} with sterile distilled water for inoculation with the aid of an Improved Neubauer hemocytometer.

2.3. In vitro test

A stock solution of BABA at 50 g L^{-1} was prepared with sterile distilled water containing 0.01% (vol/vol) Tween-20 and filtered through 0.45 μm microporous membrane filter. Potato dextrose broth (PDB) was amended by adding aliquots of the stock solution of BABA to give a final concentration of BABA at 0 (control), 0.5, 2.0 or 5.0 g L^{-1}. 100 μL of conidial suspension of *A. alternata* (5×10^6 conidia mL^{-1}) was transferred into a sterile glass tube (180 mm × 16 mm) containing 5 mL of the amended PDB. All tubes were incubated at 27 °C on a rotary shaker at 100 rpm. Approximately 200 conidia were measured for germination rate per treatment within each replicate. There were triple replicates per treatment and the experiment was performed twice.

A mycelial disc (6 mm in diameter) of *A. alternata* was prepared with a sterile iron borer from a colony cultured on PDA for 10 days at 27 °C. The disc was placed on a Petri dish (90 mm in diameter) containing 20 mL of the amended PDA medium with a final concentration of BABA at 0 (as control), 0.5, 2.0 or 5.0 g L^{-1}. The Petri dishes were incubated at 27 °C until the fungus completely covered the surface of the control Petri dishes. The colony diameter of the radial mycelial growth of *A. alternata* was measured. Each treatment contained three replicates and the experiment was repeated twice.

2.4. Inoculation and disease evaluation

Inoculation was carried out 24 h after BABA dipping. The jujube fruit (180 in each treatment containing three replicates) were surface-sterilized with 70% (vol/vol) ethanol, and wounded with a sterilized nail at two separated sites (2 mm deep × 2 mm wide) on the opposite sides of the equator surface of each fruit. Fifteen microliters of the conidial suspension (2×10^5 conidia mL^{-1}) of *A. alternata* was injected into each wounded site. Then the inoculated fruit were incubated at 15 °C, 80–90% RH for disease development. Disease incidence and lesion area on each fruit were observed and recorded after the incubation. When the visible rot zone beyond the wounded site on the fruit was more than 1 mm wide, the site was scored as an infected one.

2.5. Evaluation of natural infection

Jujubes (600 fruit in each treatment containing three replicates) were used for evaluation of natural infection during storage at 0 °C, 85–95% RH. When a spot area naturally growing mould or with brown discoloration appeared on the fruit surface was more than 2 mm wide, the fruit was counted as an infected one. Natural infection rate was expressed as a percentage (%) of the infected fruit to the total.

2.6. Measurement of fruit firmness

Thirty jujubes were used for measurement of firmness. Three measurements of flesh firmness were made on three separated but equidistant peeled sites on the equator of each fruit using a penetrometer (model GY-B, Mudanjiang Mechanical Institute, Heilongjiang, China) equipped with a flat probe (3 mm diameter). Firmness was expressed as the maximum force (N) attained during the penetration.

2.7. Biochemical assessment

Jujubes were dipped with 0 (as control) or 1.0 g L^{-1} BABA as described above and sampled for biochemical assessment during storage for 0, 15, 30, 45 and 60 days at 0 °C, 85–95% RH, respectively.

For peroxidase (POD) assay, sampled tissue (2.0 g) was homogenised on ice with 6 mL of 100 mmol L^{-1} sodium acetate buffer, pH 5.5, containing 20 g L^{-1} polyvinyl polypyrrolidine. The homogenate was centrifuged at 12 000 × *g* at 4 °C for 20 min and the supernatant was collected for the enzyme assay according to Hammerschmidt et al. (1982). The POD activity was expressed as an increase in absorbance at 470 nm min^{-1} g^{-1} fresh weight (FW).

For phenylalanine ammonia-lyase (PAL) assay, sampled tissue (2.0 g) was homogenised on ice with 4 mL of 100 mmol L^{-1} sodium borate buffer, pH 8.8, containing 1 mmol L^{-1} β-mercaptoethanol,