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Antifungal activity of 1-methylcyclopropene (1-MCP) against anthracnose (*Colletotrichum gloeosporioides*) in postharvest mango fruit and its possible mechanisms of action

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ABSTRACT

Anthracnose caused by *Colletotrichum gloeosporioides* is one of the most important postharvest diseases in mango fruit, often causing huge economic losses. In this study, the effect of 1-methylcyclopropene (1-MCP) against anthracnose in postharvest mango fruit and the mechanisms involved were investigated. 1-MCP induced reactive oxygen species (ROS) generation, damaged the mitochondria and destroyed the integrity of plasma membrane of spores of *C. gloeosporioides*, significantly suppressing spore germination and mycelial growth of *C. gloeosporioides*. 1-MCP also decreased the decay incidence and lesion expansion of mango fruit caused by *C. gloeosporioides*. For the first time this study demonstrated that 1-MCP suppressed anthracnose of postharvest mango fruit by directly inhibiting spore germination and mycelial growth of *C. gloeosporioides*, thus providing a promising strategy for disease control.

1. Introduction

Mango (*Mangifera indica* Linn.) is an important tropical fruit because of its desirable flavour and high marketing value. However, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) is one of the most serious diseases in mango fruit, attacking various parts of the mango and causing latent infection in small fruit (Kefialew and Ayalew, 2008). Application of synthetic fungicides is the major approach for control of fruit diseases. However, due to the growing concern for the environment and human health, there is an increased need to seek alternative strategies for disease control. Recently, a number of antifungal compounds, including oxalic acid (Zheng et al., 2007), salicylic acid (Tian et al., 2007), plant extracts (Regnier et al., 2008; Linde et al., 2010), calcium (Zhu et al., 2010) and borate (Shi et al., 2012), have been demonstrated to be promising strategies for controlling postharvest diseases of mango fruit.

Fleshy fruits are designated as climacteric and non-climacteric according to the ripening characteristic (Adams-Phillips et al., 2004). Climacteric fruit are characterized by a burst of respiration at the onset of ripening along with a large rise in ethylene production. Their ripening and senescence can be initiated by exposure to exogenous ethylene. 1-Methylcyclopropene (1-MCP) is a cyclic alkene that is able to bind the ethylene receptor, block ethylene action (Sisler, 2006; Watkins, 2006), and prevent the activating of senescence- and ripening-associated genes. 1-MCP has been proven to have enormous benefit in controlling ripening and senescence of a number of climacteric fruit, and maintain their quality and extend shelf life, such as apple (Bleecker and Kende, 2000; Watkins and Nock, 2012; Yang et al., 2013), banana (Baez-Sañudo et al., 2009), orange (Porat et al., 1999), strawberry (Ku et al., 1999), apricot (Fan et al., 2000), papaya (Fabi et al., 2007; Manenoi et al., 2007), tomato (Zhang et al., 2009), jujube (Zhang et al., 2012), durian (Amornputti et al., 2014) and mango (Hofman et al., 2001). Moreover, some studies also showed its important roles in inhibiting or alleviating fruit superficial scald, friction browning, skin browning and internal browning (Isidoro and Almeida, 2006; Liu et al., 2013). However, to date, most studies on 1-MCP focused only on its role in controlling ripening and senescence of fruit. Little research has demonstrated its function in controlling fungal pathogens.

In the present study, to help understand the role of 1-MCP on controlling fungal pathogens of postharvest fruit, its effect on anthracnose in postharvest mango fruit was determined, and its effect on growth of *C. gloeosporioides in vitro* was also investigated.

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2. Materials and methods

2.1. Plant material

Mango (*Mangifera indica* Linn.) fruit were harvested in the experiment orchard of Lingshui county, Hainan province, and transported to laboratory immediately where they were sorted based on size without physical injuries or infections. Selected fruit were surface-disinfected with 1% (v/v) sodium hypochlorite for 2 min, washed with tap water, and then air-dried prior to use.

2.2. 1-MCP

A weight of 1.579 g 1-MCP (0.14%, SmartFresh™ Technology) was dissolved in 10 mL distilled water, stored at 5 °C, and immediately applied to the following experiments.

2.3. Pathogen

C. gloeosporioides was isolated from decayed mango fruit and cultured on potato dextrose agar (PDA) at 25 °C for 21 d. The spores were obtained by flooding the cultures with sterile distilled water containing 0.05% (v/v) Tween-80 and adjusted to 1×10^4 spores/mL with the aid of a hemocytometer.

2.4. Effect of 1-MCP on anthracnose disease on mango fruit

Mango fruit were wounded in the middle of each fruit with a sterile nail. Each wound was then inoculated with 5 μL *C. gloeosporioides* (1×10^4 spores/mL). Half of the total fruit were put in sealed fresh-preserved storage container (volume: 9.072 L) with 453.6 μL 1-MCP solution (5 μL/L 1-MCP in the sealed fresh-preserved storage container). The half of the total fruit were put in the sealed fresh-preserved storage container with 453.6 μL distilled water and used as the control. All fruit were stored at 25 °C. Disease incidence and lesion diameter were observed. Each treatment contained three replicates of 15 fruits and the entire experiment was repeated twice.

2.5. Effect of 1-MCP on C. gloeosporioides in vitro

Aliquots of 3 μL of spore suspension of *C. gloeosporioides* (1×10^4 spores/mL) were spotted onto PDA in sealed Petri dishes (diameter: 90 mm, volume: 60 mL). Then 3 μL 1-MCP solution was spotted onto the uninoculated surface of the PDA (5 μL/L 1-MCP in the Petri dishes, respectively). The Petri dishes were immediately sealed and incubated at 25 °C. *C. gloeosporioides* with 3 μL distilled water spotted onto the PDA served as the control. The effect of 1-MCP on mycelial growth of *C. gloeosporioides* was observed. Each treatment was replicated three times and the experiment was repeated twice.

Aliquots of 5 mL potato dextrose broth (PDB) were placed in glass tubes (180 × 25 mm) with or without 5 μL/L 1-MCP. One hundred microliters of spore suspensions of *C. gloeosporioides* (1×10^4 spores/mL) was added into the glass tubes. All treated tubes were placed in a rotary shaker at 200 rpm at 25 °C. After 6, 8, 10 and 12 h incubation, approximately 300 spores of the pathogen were assessed for spore germination. Spores were considered germinated when germ tube length was equal to or greater than spore length. Each treatment was replicated three times and the experiment was repeated twice.

2.6. Effect of 1-MCP on reactive oxygen species (ROS) generation and MDA content of spores

The oxidant-sensitive probe 2′,7′-dichlorodihydrofluorescein diacetate (DCHF-DA) was used to assess the intracellular ROS levels in *C. gloeosporioides* according to the methods of Shi et al. (2012). Spores of *C. gloeosporioides* were cultured in PDB medium supplemented with or without 5 μL/L 1-MCP and incubated for 6, 12 and 18 h. Then, the spores were washed with 10 mM potassium phosphate buffer (pH 7.0), and incubated for 5 min in the same buffer containing 10 μM DCHF-DA (dissolved in dimethyl sulfoxide). After being washed twice with potassium phosphate buffer, spores were examined under a Zeiss Axioskop microscope (Carl Zeiss, Oberkochen, Germany) and the percentage of spores stained by DCHF-DA in each treatment calculated.

Spore suspensions of *C. gloeosporioides* (1×10^4 spores/mL) were added to conical flasks containing 0.1 L PDB, and the mycelium collected after culturing in a rotary shaker at 200 rpm at 25 °C for 3 d. Aliquots of mycelium (2 g) were then placed in conical flasks with 0.1 L PDB and 5 μL/L 1-MCP, and cultured in a rotary shaker at 200 rpm and 25 °C. After exposure for 0, 6, 12 and 18 h, the mycelium was collected and the MDA content was determined according to the methods of Wang et al. (2005). Mycelium (2 g) placed in a conical flask with 0.1 L PDB served as the control. There were three replications in each treatment, and the experiment was performed twice.

2.7. Effect of 1-MCP on mitochondria of spores

The spores were incubated in liquid PDB medium with or without 5 μL/L 1-MCP and incubated for 6, 12 and 18 h at 25 °C. The effect of 1-MCP on mitochondria of spores of *C. gloeosporioides* was detected according to the methods of Shi et al. (2012).

2.8. Membrane integrity assay

After treatment with 5 μL/L 1-MCP for 6, 12 and 18 h, spores of *C. gloeosporioides* were removed and stained with propidium iodide (PI). Stained spores were observed and photographed using a Zeiss Axioskop 40 microscope (Carl Zeiss, Oberkochen, Germany) equipped with an individual fluorescein rhodamine filter set (Zeiss no. 15: excitation BP 546/12 nm, emission LP 590 nm), and imaged using an Axiocam MRc digital camera (Carl Zeiss, Oberkochen, Germany). Three visual fields were randomly chosen for each sample, and the number of spores in bright field was defined as total number. Membrane integrity (MI) was calculated with the following formula: MI = [1 − (the number of spores in fluorescent field / the number of spores in bright field)] × 100%. Each treatment was replicated three times.

2.9. Statistical analysis

All statistical analyses were performed with SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) and analysed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range tests. Differences at $P = 0.05$ were considered significant.

3. Results

3.1. Effects of 1-MCP on disease incidence and severity of anthracnose

1-MCP significantly suppressed anthracnose of mango fruit caused by *C. gloeosporioides* (Fig. 1A). After 6 d, the control fruit were completely decayed, but the decay incidence of 1-MCP treated fruit was only 45.7% (Fig. 1B). In addition, after inoculation with *C. gloeosporioides* for 6 and 9 d, the lesion diameter in the 1-MCP treated fruit were only 5.67 and 15 mm, but the lesion diameter in the control fruit were 28.33 and 66 mm, respectively (Fig. 1C). These results indicated that 1-MCP treatment significantly suppressed anthracnose caused by *C. gloeosporioides* in mango fruit.

3.2. Effects of 1-MCP on mycelial growth and spore germination of C. gloeosporioides in vitro

Compared to the control, the colony diameter of *C. gloeosporioides* was significantly inhibited by 1-MCP after treatment for 96 h (Fig. 2A