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Application of a hydrothermal-calcium chloride treatment to inhibit postharvest anthracnose development in papaya

Lidia Elena Ayón-Reyna[a], Arturo González-Robles[b], José Guadalupe Rendón-Maldonado[a], María Elena Báez-Flores[a], Martha Edith López-López[a], Misael Odín Vega-García[a,*]

[a] Doctorado Regional en Biotecnología, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Cd. Universitaria, Av. de las Américas y Josefa Ortiz S/N, Culiacán, Sinaloa, 80010, Mexico
[b] Departamento de Infectómica y Patogénesis Molecular, Centro de Investigación y de Estudios Avanzados, Av. Instituto Politécnico Nacional 2508, San Pedro Zacatenco, México D.F., 07360, Mexico

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ABSTRACT

Anthracnose is considered an important postharvest disease in papaya. The hydrothermal treatment (HT) and calcium chloride (Ca) have been shown to be effective to inhibit anthracnose. The objective of this study was to investigate the effect of the combination HT-Ca on the development of anthracnose in papaya. Fruit were inoculated with *Colletotrichum gloeosporioides* by immersion in a spore suspension and then were divided into two groups: one received a HT treatment (48 °C, 20 min) combined with Ca (1% w/v, 20 min) and the other was used as control. Afterwards, fruit were stored during 20 days at 12 °C to allow the development of the fungal infection. Anthracnose incidence and severity were estimated visually while the development of the disease was analyzed by light and electron microscopy. HT-Ca reduced anthracnose incidence and severity compared with the control. Microscopy analysis showed that HT-Ca melted the epicuticular wax, which covered most of the stomata; this resulted in a lower mycelial growth in HT-Ca fruit with respect to the control samples. HT-Ca also induced the formation of round shaped vesicles, which corresponded with the greater accumulation of total phenolics observed in treated fruit. HT-Ca was effective to delay the symptoms of anthracnose up to 10 days during storage of papaya at 12 °C.

1. Introduction

Papaya (*Carica papaya* L.) is an important fruit cultivated in tropical and subtropical areas (Sharma, 2015); India is the main papaya producing country, while Mexico occupies the sixth place, providing 37.1% and 5.9% of the total production, respectively (FAOSTAT, 2014). This fruit has an important antioxidant activity and its consumption provides health benefits, particularly by its medicinal properties, and improved digestion (Chutichudet and Chutichudet, 2014). Because papaya is a climacteric fruit, it suffers some problems such as rapid ripening and susceptibility to biotic and abiotic stresses (Zhu et al., 2013). Softening related with ripening makes this kind of fruit vulnerable to a wide range of postharvest diseases including anthracnose (Ong et al., 2013), a disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. that causes great economic losses because it affects the production and marketing of papaya (Sripong et al., 2015). The symptoms of anthracnose in papaya are characterized by round brownish depressed lesions, and in some cases, salmon-colored areas formed by the conidial masses that cover the lesion (Gomes-Moraes et al., 2013). *Colletotrichum* species can directly penetrate the fruit skin or throughout openings like stomata and wounds (Gomes-Moraes et al., 2013). The incidence of anthracnose is favored by temperatures about 25–28 °C. Nevertheless, the symptoms may also appear at temperatures below 12 °C if the periods of wetness are long enough (Ferrari-Rockenbach et al., 2015).

The control of anthracnose in fresh produce is currently based on the application of chemical fungicides (Sripong et al., 2015). Nevertheless, the use of fungicides at high concentrations for long periods of time can induce resistance in the pathogen and favor environmental contamination (Shi et al., 2012). Moreover, there is concern that the fruit may be harmful to the consumers if they have fungicide residues (Ong et al., 2013). At this respect, the alternatives are expected to include both reduction of fungicides resistance and lessen the risk related to the use and abuse of

* Corresponding author.
E-mail address: mvega6@yahoo.com (M.O. Vega-García).

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fungicides to humans, animals, and the environment (Hasan et al., 2012). One strategy to improve the safety, quality and shelf-life of fruit is to identify treatments that are non-toxic, safe, biodegradable, and effective as antimicrobial agents while retaining the physical and nutritional quality (Ong et al., 2013).

There are several approved quarantine treatments for importation of papaya into the United States, such as cold treatment, vapor heat treatment, steam sterilization hot air, irradiation, and hot water immersion (USDA-APHIS, 2015). As a protocol for exportation, papayas are dipped in hot water at 48 °C for 20 min to control postharvest diseases, since hot water immersion (also called hydrothermal treatment) could directly inhibit fungal germination and growth, or even kill the fungus (Li et al., 2013b; USDA-APHIS, 2015). Li et al. (2013a) reported that hot water treatment (54 °C, 4 min) in papaya fruit reduced anthracnose incidence.

Another approach to control postharvest diseases is the use of calcium salts. It has been reported that calcium maintains cell wall structure by interacting with pectin to produce calcium pectate complexes, which can maintain fruit firmness and reduce the accessibility of fungal pathogens (Ayón-Reyna et al., 2015). Some reports have shown that calcium is antagonistic to *C. gloeosporioides* and it may be used as an alternative treatment for disease control (Ghani et al., 2011; Madani et al., 2014).

Some studies have evaluated the combination of different methods, such as hot water and calcium salts. It has been reported that hydrothermal treatment combined with calcium salts was effective to suppress microbial growth and to maintain the quality in some fresh products, such as fresh-cut papaya (Ayón-Reyna et al., 2015), fresh-cut melon (Silveira et al., 2011), apple (Sharma et al., 2013), and kiwifruit (Shahkoomahally and Ramezanian, 2013). Meanwhile, Dessalegn et al. (2013) found lower anthracnose incidence in mango fruit treated with a combination of hot water and calcium than with treatments alone.

The objective of the present study was to determine the effect of the combination of hot water and calcium chloride on the development of papaya anthracnose.

2. Materials and methods

2.1. Plant material

Maradol papaya fruit were harvested at ripening stage 4 (skin slightly orange with green stripe and pulp completely orange) (Santamaría-Basulto et al., 2009). Fruit were obtained from a local commercial plantation close to Culiacan City, State of Sinaloa, Mexico (located at 24°52′56.8″N latitude and 107°26′52.1″W longitude) and were selected based on absence of physical damage and an average weight of 1 kg. Food-grade calcium chloride (CCFO21–00 Fabpsa, Mexico) was used for the HT-Ca treatment solution (1% w/v).

2.2. Isolation and identification of fungal pathogen

Colletotrichum gloeosporioides was isolated from cv. Maradol fruit. To isolate the pathogen, infected tissue pieces were taken and placed in the center of Petri dishes containing potato dextrose agar (PDA) (MCD LAB, Tlalnepantla, Estado de Mexico, Mexico) and incubated at room temperature (25 ± 2 °C). Once mycelial growth was observed, the fungus was re-isolated on fresh PDA to obtain pure cultures (Casarrubias-Carrillo et al., 2002).

The isolates were identified on the basis of their morphological and cultural characteristics by lactophenol blue stains and according to the keys of Barnett and Hunter (1972). The fungus identity was confirmed by molecular techniques using the internal transcribed spacers ITS1-ITS2 (White et al., 1990).

2.3. Inoculum preparation

Spores were scraped from a two-week culture and placed in sterilized distilled water. Conidial suspension was adjusted to 1.5×10^5 conidia mL^{-1} and added with 0.5% Tween 80® to prevent spore clumping. Conidia were counted using a hemacytometer (Hasan et al., 2012).

2.4. Treatments application

Papaya fruit were surface-sanitized by dipping in 1% sodium hypochlorite solution for 5 min, rinsed in sterile distilled water, and inoculated by dipping into the spore suspension for 5 min (Ademe et al., 2013).

Fruit were randomly divided into two groups. One of them did not receive a treatment and was used as control, and the other group was immersed for 20 min in hot water (48 °C) containing 1% (w/v) calcium chloride, using a water bath with a temperature controller (Model 1266–02; Cole Parmer, Ill., U.S.A.). Calcium chloride concentration and hydrothermal treatment conditions were based on previous reports (Couey et al., 1984; Ayón-Reyna et al., 2015). After the application of the treatments, fruit were placed into unsealed plastic bags and stored at 12 °C for 20 days with a 90–95% relative humidity. Three replicates per treatment were performed and each treatment included 12 fruit.

2.5. Disease incidence and severity

Disease incidence was recorded according to Ong and Ali (2015) based on the anthracnose symptoms on fruit surfaces. The effect of HT-Ca on disease incidence was evaluated daily until all fruit showed symptoms. It was expressed as the number of fruit showing anthracnose out of the total number of fruit in each treatment.

Disease severity was scored at 4 day intervals following the method of Ademe et al. (2013). Disease severity was rated using a 1 to 5 scale, where 1 represented no sign of anthracnose disease on the fruit surface. A rating of 2 was scored when up to a quarter (1–25%) of the fruit surface was rotten. A rating of 3 was recorded when 26–50% of the fruit surface was infected with anthracnose. If 51–75% of the fruit surface exhibited anthracnose symptoms, a rating of 4 was scored. If anthracnose symptoms appeared on more than 76% of the fruit surface, then a rating of 5 was scored.

2.6. Light and transmission electron microscopy

Papaya samples were prepared for light and transmission electron microscopy as described by Phothiset and Charoenrein (2013). Sections of about 2 × 2 × 2 mm were excided from the fruit surface at 0, 12, and 24 h, and at 2, 4, 5, 6, 8, 10 and 12 days after the treatments were applied and then transferred to 1.5 mL microtubes containing 2.5% glutaraldehyde in 0.1 mol L^{-1} sodium cacodylate buffer (pH 7.4), followed by incubation for 12 h at room temperature. Samples were rinsed three times with the same buffer for 20 min and post-fixed with 1% v/v osmium tetroxide in 0.1 mol L^{-1} sodium cacodylate buffer (pH 7.4) for 2 h at room temperature. Samples were dehydrated in an ascending ethanol series (30, 50, 70, 80, 90, and 100%) for 20 min each. To complete the dehydration, they were immersed twice in absolute ethanol for 20 min each. Afterwards, dehydrated samples were rinsed in propylene oxide (three times, 20 min each), followed by infiltration overnight in a mixture of propylene oxide–polybed resin in a 1:1 ratio, then they were transferred to propylene oxide–polybed resin mixture in a 1:2 ratio followed by an infiltration in pure polybed resin three times, for 1.5 h each. Later on, for polymerization, they were placed in an oven at 60 °C for 48 h and sectioned with a