Infection Assays of Tomato and Apple Fruit by the Fungal Pathogen *Botrytis cinerea*

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[Abstract] *Botrytis cinerea* (*B. cinerea*) is an aggressive fungal pathogen that infects more than 200 plant species. Furthermore, the pathogen can attack fruits of some plants, such as tomato and apple. *B. cinerea* has become one of the model systems in molecular phytopathology because of its economic importance and sophisticated genetic operation methods. Virulence assays are very important in the study of fungal pathogenesis. This protocol details the artificial inoculation procedure of *B. cinerea* on tomato and apple fruits. It also can be used to analyse the virulence of postharvest fungal pathogens on other fruits, such as pear, peach, jujube and so on.

Materials and Reagents

1. Freshly harvested tomatoes and apples (fully ripe)
2. *Botrytis cinerea* (B05.10): Supplied by Prof. Paul Tudzynski Westfaelische Wilhelms-Universitaet Muenster, Germany
3. KH_2PO_4 (Beijing Chemical Works, catalog number: HG/T 1274-1993)
4. Glucose (Beijing Chemical Works, catalog number: HG/T 3475-1999)
5. 2% sodium hypochlorite (Xilong Chemical, catalog number: 7681-52-9)
6. Potato
7. Dextrose
8. PDA medium (see Recipes)
9. 2% sodium hypochlorite (see Recipes)
10. KH_2PO_4-glucose solution (see Recipes)

Equipment

1. Glass stick
2. Hemacytometer (QIUJING, model: XB-K-25)
3. Cheesecloth
4. Vortexer (Select BioProducts, model: SBS100-2)
5. Optical microscope (Chongqing Optec Instrument, model: B203LED)
6. Clean bench (Donglian Electronic & Technology Development, model: SCB-1520)

7. Pipette
8. Plastic basket
9. Sterile nail (approximately 3 mm in diameter)

Procedure

A. Fruit disinfection
1. The fruits with uniformity and without physical injuries were used as experimental materials.
2. These fruits were immersed in 2% sodium hypochlorite solution for 2 min, rinsed with sterile tap water, and air-dried in a clean bench (approximately 2 h).
3. After being air dried, the fruits were wounded (approximately 4 mm in depth) at the equator with sterile nail (sterilized with alcohol lamp) prior to inoculation with pathogen (Figure 1).

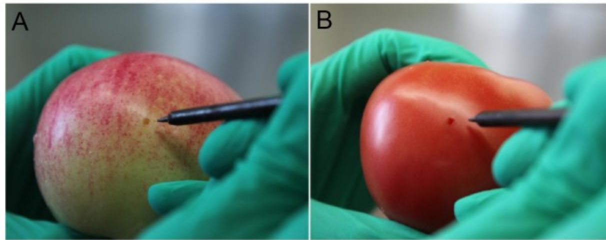

Figure 1. Wounding of apple fruit A and tomato fruit B with sterile nail

B. Pathogen inoculum preparation
1. Five microliter spore suspension (5 x 10^6/ml in 15% glycerol, stored at -80 °C) of *B. cinerea* was inoculated on PDA plates and cultured for 2 weeks at 22 °C.
2. Fungal spores were harvested by flooding the surface of the culture with KH_2PO_4-glucose solution containing 0.05% (v/v) Tween-80, then filtered through four layers of sterile cheesecloth. The concentration of spore suspension was determined by observing under optical microscope using hemacytometer. Then, the concentration of spore suspension was adjusted to 5 x 10^4 per milliliter by KH_2PO_4-Glucose solution.

C. Inoculation

Tomato and apple fruits were inoculated with 10 μl spore suspension (the suspension was mixed by Vortexer before inoculation) in each wounded site using a pipette, respectively, KH_2PO_4-Glucose solution was used as control. Ten inoculated fruits were put into plastic basket (40 cm x 30 cm x 10 cm) and sealed with plastic film, about 5mL sterile water was

sprayed into the plastic film to maintain a high relative humidity (about 95%), and stored at 25 °C.

D. Disease scoring

Two days after inoculation, the lesion diameters were measured daily. Two diameter values of each lesion in two mutually perpendicular directions were recorded. The average of the two values was defined as the diameter of the lesion (Figure 2).

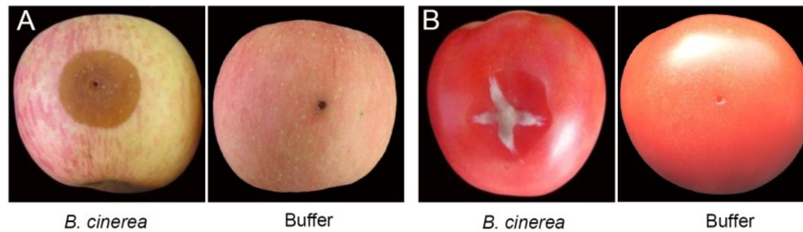

Figure 2. Lesions caused by *B. cinerea* and buffer. A. lesions on apple fruits (four days after inoculation). B. lesions on tomato fruits (3 days after inoculation). Buffer, KH_2PO_4-Glucose solution. (Source: Zhang *et al*., 2014)

Recipes

1. PDA medium (1 L)
   Potato 200 g
   Dextrose 20 g
   Agar 15 g
   Boiling 200 g of sliced potatoes in 1 L distilled water for 30 min, then decanting the broth through cheesecloth and adding 20 g dextrose and 15 g agar powder in the broth. Add distilled water to make up 1 L, and the medium is sterilized by autoclaving for 20 min.
2. 2% sodium hypochlorite (5 L)
   100 ml sodium hypochlorite
   4.9 L dH_2O
3. KH_2PO_4-glucose solution (100 ml)
   KH_2PO_4 0.136 g (10 mM)
   Glucose 0.198 g (10 mM)
   Tween-80 50 µl (0.05%, v/v)

Acknowledgments

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