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Dry Heat Treatment Reduces the Occurrence of *Cladosporium cucumerinum*, *Ascochyta citrullina*, and *Colletotrichum orbiculare* on the Surface and Interior of Cucumber Seeds

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Abstract

Dry heat treatment has been identified as a method for disinfecting seed-borne pathogens in vegetable seeds. This study demonstrated that three seed-borne pathogens of cucumber (*Cladosporium cucumerinum* that causes scabs, *Ascochyta citrullina* that results in gummy stem blight, and *Colletotrichum orbiculare* that induces anthracnose) could be effectively eradicated from cucumber seeds by dry heat treatment. *In vitro* growth of these three pathogens was inhibited by dry heat treatment at 70 °C for 40 min. These pathogens were inactivated after exposing infected seeds to 70 °C dry heat for at least 90 min. Seed infection was significantly reduced by exposing the seeds to 70 °C dry heat for at least 40 min. Seed moisture content and germination were slightly reduced after 70 °C heat treatment for 40–120 min. Seed vigor remained at a high level after dry heat treatment at 70 °C for 90 min. In conclusion, 70 °C dry heat treatment for 90 min was determined to be the optimal method for eradication of *C. cucumerinum*, *Didymella bryoniae*, and *C. orbiculare* from cucumber seeds.

Keywords: dry heat treatment; *Cladosporium cucumerinum*; *Ascochyta citrullina*; *Colletotrichum orbiculare*; scab; gummy stem blight; anthracnose

1. Introduction

Cucumber (*Cucumis sativus* L.), a member of the Curcurbitaceae, is one of the most common vegetables in China. Cucumber scab, cucumber gummy stem blight, and cucumber anthracnose, which are caused by *Cladosporium cucumerinum*, *Ascochyta citrullina*, and *Colletotrichum orbiculare*, respectively, are three economically significant diseases affecting cucumber production. These pathogens are capable of surviving from season to season inside or on the surface of cucumber seeds (Du et al., 1984; Zhang et al., 2006; Nasreen et al., 2009). Diseased plants, seeds, weeds, wild cucurbits, and infected soil debris are all potential primary sources of *C. cucumerinum*, which can be found on the surface of or inside seeds the next year, thereby resulting in a high infection rate in cucumber plants (Yuan et al., 1991). *A. citrullina* is often detected in the seed perisperm and cotyledons (Du et al., 1984). Therefore, seed treatment schemes that effectively eliminate pathogens yet impart minimal to negligible seed damage are warranted.

Physical, chemical, and biological seed treatments have been proven effective for seed disinfection (Kritzman, 1993; Thomas and Adcock, 2004; Sajid et al., 2006; Schmitt et al., 2009). However, chemical seed treatment using a single chemical reagent cannot consistently reduce pathogen populations (Taormina et al., 1999). Fungicide seed treatments reduce the transmission frequency of anthracnose, but do not eradicate pathogens inside the seeds (Thomas and Sweetingham, 2003). Dry heat treatment, which has been developed several years ago, is a common physical treatment for seeds and has been applied to various vegetables, including cucurbits (Masaharu et al., 2012), solanaceous crops (Li et al., 2011), *Brassica* (Song et al., 2011), lettuce (Schmitt et al., 2009), spinach (Dadali et al., 2007), and carrots (Bang

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et al., 2011). This method can completely inactivate noxious seed-borne bacterial pathogens such as *Erwinia*, fungal pathogens such as *Fusarium*, *Alternaria*, and *Cladosporium* (Jung, 2004), as well as the cucumber green mottle mosaic virus (Kim and Lee, 2000).

The present study analyzed the relationship between the conditions of dry heat treatment and the effectiveness of disinfection of seeds infected by *C. cucumerinum*, *A. citrullina*, and *C. orbiculare*.

2. Materials and methods

2.1. Collection of seed samples

The crop surveys were conducted in Shandong and Liaoning Provinces in China from August to October 2013. Plants grown in Wafangdian, Liaoning Province were diagnosed to have scab based on typical symptoms. Field or greenhouse plants in Weifang, Shandong Province were diagnosed to be infected with gummy stem blight and anthracnose. Initially, the pathogen *D. bryoniae* was expressed on leaves in the form of angular lesions, which subsequently dry up and drop off, thereby resulting in ragged lesions. Symptoms of anthracnose infection include circular spots on leaves, whereas more severe cases involve leaf desiccation. The stem base changes color to yellowish-brown and shrinks. Cucumber leaves presenting typical disease symptoms caused by different pathogens were collected. Small pieces of tissue that were cut from the margins of lesions were disinfected in 1% NaClO for 3 min, rinsed three times with sterile water, and placed on potato dextrose agar (PDA) plates. Conidia were harvested from 14-day-old cultures grown on PDA under a 12 h light/12 h dark photoperiod using near-ultraviolet light at 25 °C. Images of the microstructures of conidia and conidium terrier were captured. Cucumber seeds were extracted from rotten fruits collected from the provinces and air-dried for 4 days. The seeds were then dried in ovens at 40 °C for 3 days and stored at 4 °C in polythene bags. The seed cultivars used in the present study were 'Jinyou 36' (J36), 'Jinyou 35' (J35), and 'Jinyou 12' (J12). Seeds of similar size, color, and shape were selected for the subsequent tests, which were conducted in triplicate.

2.2. Isolation of pathogens and pathogenicity testing

Approximately 500 g of seeds that were infested with *C. cucumerinum, D. bryoniae*, and *C. orbiculare* were respectively collected from infected rotten fruits of cultivars J36, J35, and J12. Symptomatic plant parts isolated from different zones were vortexed in 75% ethyl alcohol for 30 s and in 1% sodium hypochlorite (NaClO) solution for 5 min, and then rinsed three times with sterile distilled water. Cultures of each pathogen were inoculated onto new dishes of PDA medium (20 mL in a 90 mm triple-vented Petri dish) and incubated in the dark at 28 °C for 11 days (International Seed Testing Association, 1981).

To confirm pathogenicity of the *C. cucumerinum*, *D. bryoniae*, and *C. orbiculare* isolates from J36, J35, and J12, 30-day-old cucumber plants were randomly selected and inoculated at the collar region with a spore suspension at a density of 3.0×10^5 in sterile distilled water. Plants that were inoculated with sterile distilled water served as controls. The plants were covered with plastic bags for 2 days and kept at 23–25 °C and 90% relative humidity, with a 12 h photoperiod under greenhouse conditions. Plants were assessed for disease from 7 to 30 days post-inoculation. Experiments were conducted in four replicates of 10 plants each and repeated three times for all three isolates.

2.3. Effects of dry heat treatment on in vitro fungal colony growth

For each of the three pathogens, circular plugs (5 mm in diameter) were cut from non-sporulating mycelia from 7-day-old culture dishes using a cork-borer, and a single plug was placed upside down in the center of a new dish containing culture medium (sterile gauze in a 90 mm triple-vented Petri dish). The dishes were respectively subjected to temperature treatments of 55, 60, 65, and 70 °C for 20, 40, 60, 90, and 120 min using a hot air oven (MEMMERT Universal oven). The hot air oven was heated evenly and the temperature had a setting accuracy of 0.1 °C.

After heat treatment, a single plug from the treated plate was placed upside down in the center of a new dish containing culture medium (20 mL of PDA in a 90 mm triple-vented Petri dish). Dishes were sealed with Parafilm M® and incubated in the dark in incubators at 28 °C. Three replicate dishes were prepared for each strain. External radial growth was recorded after 7 days using two cardinal diameters that were previously drawn on the bottom of the dish (Helen et al., 2003).

2.4. Measurement of seed moisture content, germination, and seed vigor index (SVI)

A total of 50 cucumber seeds were used in the determination of seed moisture content using a moisture meter (OHAUS Instruction Manual MB45 Moisture Analyzer), and each treatment was replicated four times.

Approximately 100 cucumber seeds were placed on moist sterile filter papers in 90 mm Petri dishes for 12 h to monitor germination, and each treatment was replicated four times. The emergence of 2 mm buds was scored, and the percentage of germination was calculated using the following formula (Copeland and McDonald, 1995): Germination (%) = (number of seeds germinated/total number of seeds) × 100.

Around 50 seeds were sown in culture trays (10 cm × 10 cm) kept in a greenhouse and watered daily to determine SVI, and each treatment was replicated four times. The temperature of the greenhouse ranged from 23 °C to 30 °C, and humidity ranged from 40% to 70%. Sunlight in the greenhouse was present for 9 to 12 h each day. The average length of the seedlings was measured 2 weeks later. SVI was calculated using the following formula (Schelin et al., 2004):

$$\mathrm{SVI} = S \times \sum \frac{Gt}{Dt}$$

where *Gt* is the number of germinated seeds, *Dt* is the number of days of germination, and *S* is the average length of the seedlings.

2.5. Biological activity of pathogens on the surface and inside the seeds

To assess the biological activity of the pathogens on the surface of the seeds, four replicates of 50 seeds each were used in the