

Histological Studies of *Colletotrichum orbiculare* on the Susceptible and Resistant Cucumber Cultivars

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Abstract

The infection process of *Colletotrichum orbiculare* on the leaves of susceptible and resistant cucumber cultivars were studied histologically. On both cultivars, conidia began to germinate 8 h after inoculation (hai) and formed appressoria. Then melanised appressoria formed penetration pegs at 10 hai. On the susceptible cultivar, infection vesicles formed within 24 hai and developed thick, knotted primary hyphae at 48 hai. By 72 hai, *C. orbiculare* produced highly branched secondary hyphae that invaded underlying mesophyll cells. While on the resistant cultivar, fewer germinated conidia developed biotrophic primary and invasive necrotrophic secondary hyphae than on the susceptible cultivar. These results show that the early stages of penetration process on both cultivars have no differences, and that resistance in cucumber restricts colonization by inhibiting the development of biotrophic primary hyphae and necrotrophic secondary hyphae.

Keywords: *Colletotrichum orbiculare*, cucumber, infection process, hemibiotrophic

Introduction

Colletotrichum orbiculare, belonging to ascomycete, induces fatal anthracnose disease on cucumber (Pain et al. 1992), and has become a limiting factor in commercial production (Bi et al. 2007). The cucumber plants, both in the greenhouse and field, caused roughly circular, brown to reddish lesions on all above-ground tissues including leaves, stems, petioles, and fruits after attack by *C. orbiculare* (Langston Jr et al. 1999).

Colletotrichum species have been utilized to study fungal distinction and plant-fungi interaction for many years (Perfect et al. 1999). During the colonization on plants, a series of particular structures containing germ tubes, appressoria, penetration pegs, infection vesicles, intracellular hyphae and secondary necrotrophical hyphae are developed by *Colletotrichum*, and *Colletotrichum* displayed two nutrition modes that are biotrophic and

necrotrophy, respectively. *Colletotrichum* hence represents a splendid mode for studying molecular and cellular mechanisms of pathogenic fungi (Bailey and Jeger 1992; Perfect et al. 1999).

Previous research has demonstrated that the infection process of *C. orbiculare* on cucumber leaves is initiated by conidial adhesion, germination, appressorial development and growth of infectious hyphae (Jeun and Lee 2005; Jeun et al. 2008). However, the differences of infection process of *C. orbiculare* in susceptible and resistant cucumber cultivars are not well described. The objective of this study, therefore, was to elucidate the differences of the infection process and strategy adopted by *C. orbiculare* on two cucumber cultivars varying in resistance to the pathogen.

Materials and Methods

Pathogen, plants and inoculation

Colletotrichum orbiculare used in this study was isolated from naturally infected field-grown cucumber (Yangling, Shaanxi Province, China) and stocked in our lab. The isolated fungus was cultured on potato dextrose agar (PDA) at 25°C. When the fungus took the shape of pink conidial pile, conidial suspensions were prepared by flooding 10-day-old culture plates with 4–5 mL of sterile distilled water, gently scraping the colony surfaces with a scalpel and filtering the suspension through sterile cheesecloth. The concentration was adjusted to 1×10^6 conidia/mL using a haemocytometer.

The cucumber (*Cucumis sativus* L.) cv. 'Changchun Thorn' (susceptible) and 'Zao qing No. 2' (resistant) were obtained from the College of Plant Protection, Northwest A&F University. The cucumber seeds were each sown in plastic pots of 20 cm in diameter filled with soil. Cucumber seedlings were grown in a growth chamber at adequate moisture with a photoperiod of 12L:12D (60 mmol/m²/s photon flux density) at 25°C.

The inoculated cucumber leaves were incubated at 25°C and 100% relative humidity in the dark for 24 h

followed by incubation at 25°C, 60–70% relative humidity and a photoperiod of 12L:12D) (60 mmol/m²/s photon flux density).

Sampling and observation

Leaf samples were cut into 1 cm² segments at 24, 48, 72, 96, 120, 144 hours after inoculation (hai). Then the samples were decolorized in boiling 95% ethanol for 10 min, cleared in saturated chloral hydrate overnight, and rinsed in distilled water for 5 min.

For light microscopy, the cleared leaf segments were stained with 0.05% (w/v) Coomassie Brilliant Blue (Bio Basic Inc., Shanghai, China) for 10 min. After staining, the tissues were rinsed in distilled water for 3 min, temporarily mounted in 50% glycerol on glass slides and examined under a Nikon 50i microscope (Tokyo, Japan) equipped with a differential interference contrast (Warwar and Dickman) optics. Percentages were calculated from 60 penetration sites on each of five cleared leaf pieces. All experiments were repeated three times.

Results

The early stage of penetration of *C. orbiculare* on the leaves of susceptible and resistant cultivars

Conidia of *C. orbiculare* were oval with a smooth surface and began to germinate at 8 hai on both cucumber cultivars, less than 10% of conidia had attached to and germinated on the leaf surface of susceptible and resistant cultivars. The germination of *C. orbiculare* was judged by the formation of germ tubes or the direct generation of appressoria. About 70% conidia had germinated at 12 hai and over 90% germinated conidia had formed on both cultivars at 24 hai. The germinated conidia began to form melanised appressoria from 8 hai on susceptible and resistant cultivars. Nearly all of the germinated spores formed melanized appressoria at 12 hai and 24 hai. Melanised appressoria were formed either directly from one tip of a conidium or from the tip of a germ tube. About 8% penetration pegs were observed within 10 hai on both cultivars. Over 30% melanized appressoria had formed penetration pegs on both cultivars simultaneously at 12 hai. By 24 hai, over 50% melanized appressoria had formed penetration pegs on both cultivars.

The penetration process of *C. orbiculare* on the leaves of susceptible and resistant cultivars

By 24 hai, the swollen and saccate infection vesicles beneath the penetration pegs were observed on both cultivars. The infection vesicles with elongated neck regions had formed in epidermal cells. Primary hyphae had formed and successfully invaded the epidermal cells at 48 hai. The primary hyphae, large-diameter and lengthy but noticeably constricted at the sites of the intercellular

penetration, had expanded to develop secondary hyphae by penetrating adjacent epidermal and mesophyll cells at 72 hai on susceptible cultivar. The narrow secondary hyphae secreted cell-wall-degrading enzymes in advance of their spread and rapidly created expanding necrotic lesions. A large number of sinuous secondary hyphae had formed at 96 hai on susceptible cultivar, while the infection vesicles enlarged and formed primary hyphae and, less frequently, a few of secondary hyphae on resistant cultivar. By 120 hai, many colonies and clusters of conidia were observed on the surface of susceptible cucumber leaves, only a small number of conidia had produced secondary hyphae on resistant cultivar, significantly fewer than on the susceptible cultivar.

Discussion

Our experiment revealed that the infection process in the cucumber-*C. orbiculare* interaction is generally similar to those of other *Colletotrichum* spp. on various hosts (Ge and Guest 2011). The infection sequence of *C. orbiculare* includes conidial germination, formation of melanised appressoria and penetration pegs, then penetrate to form infection vesicles, primary hyphae, and finally necrotrophic secondary hyphae, which is similar in susceptible and resistant cultivars, like *C. lindemuthianum* on bean (O'Connell et al. 1985).

Conidia germinated and formed appressoria within 8 h after inoculation. This is earlier than *C. lindemuthianum* on French bean (O'Connell et al. 1985) or cowpea (Bailey et al. 1990). Adhesion has been implied to be required for spore germination (Kuo and Hoch 1996; Warwar and Dickman 1996; Shaw et al. 2006). Spore adhesion to the substratum is beneficial to offering the spore stability and time to receive stimuli necessary for spore germination and appressorial formation ultimately. Furthermore, it is reported that the physical and chemical structures of the epicuticular wax can also influence spore germination (Prusky and Plumbley 1992). Thus the different affinities of leaves to water may also contribute to the easier germination of *C. orbiculare* on host leaves.

Early studies indicate that the formation of large intracellular primary hyphae and necrotrophic secondary hyphae are typical characteristics of success infection with hemibiotrophic species of *Colletotrichum* (Perfect et al. 1999; Prusky and Plumbley 1992). Although almost all conidia formed appressoria on both cultivars by 24 hai, fewer developed primary and secondary hyphae on resistant cultivar. These observations suggest that the pathogen establishes the initial penetration phase rapidly in both leaves, colonises after developing invasive necrotrophic secondary hyphae in susceptible leaves,

while resistance mechanisms inhibit the transition to necrotrophy in resistant leaves, significantly. A previous study indicated that the determinants of the compatibility or incompatibility of host pathogen did not operate during the prepenetration phase of *C. gloeosporioides* f. sp. *malvae*, but was activated a few days after successful penetration (Morin et al. 1996). This point of view is supported by our present research. After the same symptom on penetration phase, studies in the *C. orbiculare*-melon interaction also discovered that infection structures transformed into a destructive, necrotrophic phase in the susceptible cultivar (Ge and Guest 2011). The reason may be linked with the secretion of depolymerases that degrade plant cell walls (Münch et al. 2008). The detailed mechanisms of the resistance in the *C. orbiculare*-cucumber interaction remain to be studied.

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