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Infection of *Colletotrichum gloeosporioides* on Mango Fruit and its Resistance

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Abstract

Infection of *Colletotrichum gloeosporioides* on mango fruit cv. Nam Dok Mai was studied. Under an optimum condition (95-100%RH, 25 °C), germination and appressorium formation started at 12 hrs and 14 hrs after deposition of conidia on the peel, respectively. After 48 hrs, 60% fungal propagules present was appressoria. The fungus infected unripe mango fruit in latent fashion. Appressorium produced an infection peg to penetrate fruit peel accompanying the maceration of cuticle. The symptoms expressed at fruit ripening and the fungus reproduced conidia by sporulating of acervulus. At postharvest stages, mango cv. Nam Dok Mai and Nang Klang Wan were found to be susceptible to anthracnose whereas Rad was resistant.

Introduction

Colletotrichum gloeosporioides is the causal agent of anthracnose, a major postharvest disease of mangoes in the tropics. The fungus infected mango fruits in latent fashion Fruit-pathogen interactions depended on expression of resistance factors by the mango cultivar and pathogenicity factors by the fungus during different periods of fruit life. For the fungus, preharvest infection remained quiescent until fruit ripen (Simmonds, 1941). For the mango fruit after harvest, resistance mechanism was related to the decrease of a mixture of antifungal compounds present in the peel (Droby *et al.*, 1987). In Thailand, many commercial mango cultivars were reported to be susceptible to the disease (Sangchote, 1987). In this paper, an infection of *C. gloeosporioides* on mango fruit and the resistance of some mango cultivars to the disease were investigated and discussed.

Materials and Methods

1. Microscopic infection of mango fruit cv. Nam Dok Mai by *Colletotrichum gloeosporioides*

C. gloeosporioides was isolated on infected mango fruit cv. Nam Dok Mai from Petchabun, Thailand, and maintained on Potato Dextrose Agar in petri dishes. Fungal inoculum for inoculation were obtained from 7-days-old colonies by suspending conidia in sterile water (for light microscopy [LM] and transmission electron microscopy [TEM]) or solution of 0.25% NaCl (for scanning electron microscolpy [SEM]). Unripe mango fruits cv. Nam Dok Mai were sprayed conidial suspension, adjusted to 250-300 conidia per 1 mm^2, then incubated in 95-100%RH at 25 °C. Peel specimens were cut from inoculated fruits during incubation.

For SEM, specimens (4x4x1 mm) were sampled at 48 hrs, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer and then dehydrated in alcohol series. Specimens were critical-point dried in CO_2 liquid, mounted on aluminum stubs, and sputtercoated with gold. Observation were made under JOEL JSM-5410LV scanning electron microscope.

For LM, specimens (4x4x0.2 mm) were samples at 2 hrs interval and immediately soaked in alcohol absolute for 4 hrs. Then the specimens were stained with cotton blue, mounted peel side up on glass slide, covered with cover-slip and observed under a light microscope.

For TEM, specimens (1x2x1 mm) were sampled at 98 hrs, fixed like for SEM and additionally in OsO_4 1%. Specimens were dehydrated in alcohol series, infiltrated and embedded in pure spur blocks. Thick sections (1 µm) were observed under light microscope (LM). Ultrathin sections (60 nm), collected on slot grids, were stained with lead citrate. Observation were made under JEOL JEM-1220 transmission electron microscope.

2. Fruit resistance of mango to anthracnose disease

The experiment included five mango trees 7-10 years old of 5 cultivars, Nang Klang Wan (NKW), Nam Dok Mai (NDM), Chok Anan (CAN), Kaew and Rad. The experiment was a completely randomized design with 3 replicates on each tree, 20 fruits and 10 fruits per replicate in 2001 and 2002 for each cultivar, respectively. The treatment design was a factorial of cultivars and fruit treatments (non-inoculated or inoculated). Each fruit was inoculated with *C. gloeosporioides* 106 conidia/ml (100 conidia per mm square of hemacytometer) on one side, the other side was non-inoculated. Inoculation was done 24 hrs from harvest for mangoes in 2001 and 6 hrs from harvest for mangoes in 2002. Inoculated fruits were incubated in

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moist plastic bags for 48 hrs then treated with ethylene 10 µL/L, and ripened in room condition. Anthracnose severity (percentage of decayed area) and incidence (percentage of decayed fruits) by postharvest inoculation were assessed at ripening stage of fruits. The severity value on non-inoculated sides were used as covariates in the model of ANOCOVA.

Results and Discussion

1. Microscopic infection of mango fruit cv. Nam Dok Mai by *C. gloeosporioides* (Figure 1)

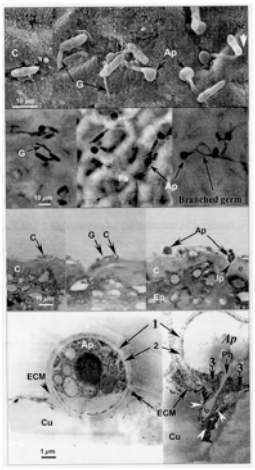

Figure 1 Infection of *C. gloeosporioides* on mango fruit.

Conidia of *C. gloeosporioides* (C) after deposition on surface of unripe mango fruit cv. Nam Dok Mai, in 95-100%RH at 25 °C, would germinate at 12 hrs. Deposition of conidia resulted in 'scars' (Sc) on epicuticular wax. One conidium could produce one or two germ tubes (G). After 48hrs, branching of the elongated germ tubes was common.

Appressorium (Ap) could be formed from a swollen tip of a simple or branched germ tube. Maturation of appressoria was defined by the appearance of cell wall melanization in dark color. The first appressorium formation was visible at 14 hrs.

The extracellular matrix (ECM) covered and supported the adherence of the appressorium to fruit surface. The appressorial wall consisted of a higher electron-opaque outer layer (1), a lower electron-opaque inner one (2), and a third wall layer forming a thickened ring around the penetration hole (3), like Wharton (2001) illustrated those of *C. sublineonlum* infecting *Sorghum bicolor*. From the appressorial penetration hole, the infection peg penetrated of the fruit accompanying the maceration of cuticle. The maceration appeared around and even more beneath the infection peg towards the epidermis (white arrowheads).

No anthracnose symptoms appeared on unripe mangoes. Subcuticular hyphae was suggested to be present during latent period of the fungus on unripe fruits (Simmonds, 1941). At ripe stage, the fungus reactivated, developed and ruptured the fruit cuticle, exposed the acervulus with conidia in salmon mucilaginous matrix.

2. Fruit resistance of mango to anthracnose disease (Figure 2)

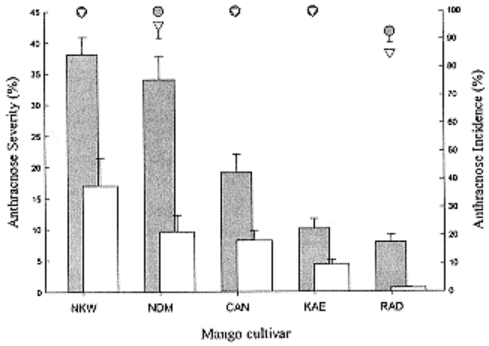

Figure 2 Anthracnose severity and incidence by postharvest inoculation on 5 mango cultivars harvested from Suphanburi Crop Research Institute in 2001 and 2002.

Anthracnose severity by postharvest inoculation for 5 cultivars were correlated (r=0.91) between years. Mango cv. Nam Dok Mai and Nang Klang Wan were susceptible to anthracnose whereas Rad was resistant. During 2001 and 2002, a survey on postharvest mangoes from Talaad Thai wholesale market (un-published data) also showed the resistance of Rad by comparing with some commercial mango cultivars.

Conclusion

C.gloeosporioides infects unripe mango fruit in latent fashion. Conidium germinates on fruit surface and produces germ tubes or saprophytic hypha whose tips will swell and form appressoria. Appressorium produced an infection peg to penetrate fruit peel accompanying the maceration of cuticle. No further development have been seen until fruit ripen, when the fungus decay the fruit and reproduce conidia by sporulating of acervulus. At ripe stages, mango cv. Nam Dok Mai and Nang Klang Wan were found to be susceptible to anthracnose whereas Rad was resistance.

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