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LIGHT AND SCANNING ELECTRON MICROSCOPY ON *Colletotrichum capsici* – A FRUIT ROT PATHOGEN AND INTERACTION WITH A SUSCEPTIBLE CHILLI VARIETY

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ABSTRACT

Rahman MME, Rahman ML, Ali MS, Islam R, Nessa M (2014) Light and scanning electron microscopy on *Colletotrichum capsici* - a fruit rot pathogen and interaction with a susceptible chilli variety. *Int. J. Expt. Agric.* 4(1), 22-25.

Light and scanning electron microscopy was done on fruit rot pathogen *Colletotrichum capsici* for a susceptible chilli (*Capsicum annuum*) variety in relation to rapid pathogenesis and host pathogen interaction. It was observed that the pathogen produced spherical, hemispherical and irregular acervuli 100-180 µm in diameter which was associated with many conspicuous setae of 93-110 µm length. Each acervulus was filled up with numerous curve conidia with 18-25 µm length. After inoculation conidia of the pathogen adhered intimately on the curved fruit surface and germinated within 24 hours to produce germ tube and formed sub-globose appressoria for initiating infection. Infected host cells became discoloured or dead in addition to extensive degradation of cell wall. Protruding fruiting bodies of the pathogen caused easy disruption of epidermal layers of host tissues.

Key words: *chilli, fruit rot, Colletotrichum, scanning electron microscopy*

INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important spice as well as cash crop in Bangladesh covering an area of 220 thousand acres of land (BBS 2010).The national average yield of Chilli in Bangladesh is low (1.12 ton/ha) as compared to that of other countries (BBS 2007). Various biotic and abiotic factors are responsible for the lower yield of the crop. Among the biotic factors diseases play a vital role to reduce the yield of chilli in the country. Many pathogens causing diseases of chilli have been reported in the country (Talukdar 1974). Among them *Colletotrichum capsici* causing die back and fruit rot is considered as important one. Fruit rot is conspicuous as it causes severe damage to mature fruits in the fields as well as in storage and transport (Singh 2004). This pathogen infects most of the plant parts including root, stem, leaf, flower and fruit (Bailey and Jeger, 1992). Investigations have been done on chemical control, morphological variation, integrated control measure, etc. of the pathogen. But, knowledge of infection process in terms of host-pathogen interaction to provide epidemiological information is necessary for preparing forecasting models (Bailey and Jeger, 1992). On the other hand a chilli variety namely BARI lanka-1 was recently found to be highly susceptible to the disease (personal communication). It was seemed that there might have many reasons including fruit morpho-physiological characteristics. Electron microscopic study on fruit rot pathogen of chilli has yet not been done in the country which may pave the way of investigation related to the rapid pathogenesis of the fungus attacking the susceptible chilli variety. Therefore, the present study was undertaken to generate information on interaction regarding pathogenesis and rapid infection process of *C. capsici* especially on the fruit of a susceptible chilli variety through light and scanning electron microscopic study.

MATERIALS AND METHODS

Light microscope and scanning electron microscope (SEM) of Plant Pathology Division, Bangladesh Agricultural Research Institute were used in the investigation. The study included sample collection and preservation, detection of inoculum source, inoculum preparation, and inoculation on chilli fruit and finally observation with light microscope and scanning electron microscope.

Inoculum source: Infected and healthy fruits of a susceptible chilli variety (BARI lanka-1) were collected from experimental fields of BARI. Samples were cleaned, processed and finally preserved in a refrigerator at 5^{o}C for further study. Sign and symptoms of infected chilli fruits were studied with the help of a light microscope. Acervuli produced by the pathogen including host tissue were observed through stereo-microscope and compound microscope. Images were taken by scanning electron microscope for clear and detail study relate to the infection process of the pathogen.

Inoculum preparation: The pathogen *C. capsici* was isolated from infected fruit on sterilized potato dextrose agar (PDA) medium. The isolated pathogen was transferred to new PDA plate and allowed to grow for 7 days. The conidia and mycelia were harvested from 7 days old culture by rinsing with sterilized water and rubbing gently with a clean brush that helped the conidia and mycelial fragments easy release for better harvest. The suspension was sieved to remove agar lumps and blended for 1.5 minutes with the help of a mechanical rotary blender which culminated the fungal suspension.

Fruit inoculation: Fruits of chilli were surface sterilized with 10% Clorox and washed repeatedly with distilled water. The fruits were inoculated with the pathogen *C. capsici* by placing single drop of fungal suspension that

smeared over the fruit surface (1 cm^2). Inoculated fruits were incubated at 20±2°C in an incubator for initiating infection.

SEM observation: Samples for SEM observation were prepared following the method of Hayat (1981). Both healthy and diseased surface of chilli fruits with acervuli were observed under SEM. In addition, after 24 hrs of artificial inoculation of the pathogen, specimens were taken for microscopic study. The specimens were dehydrated under ambient condition and adhered onto aluminium specimen mounts with sticky carbon tape. Then the specimens were coated with platinum for 30 sec through a vacuum evaporator and photographs were taken with the help of JEOL SEM (JSM6490 LA). Data on morphomatrix of acervuli, setae and conidia of the pathogen grown on PDA were also recorded under the SEM.

RESULTS AND DISCUSSION

Morphomatrix of fruiting bodies: Morphomatrical study on *Colletotrichum capsici,* the die back and fruit rot pathogen of chilli was done with the help of scanning electron microscope. The acervuli developed by the fungus on potato dextrose agar (PDA) belonged broadly to three different shapes, spherical, hemispherical and irregular (Table 1 and Fig. 1). The fungus developed numerous acervuli on PDA and majority of acervuli were hemispherical in shape. The intensity of spherical as well as irregular shaped acervuli was comparatively lower. The diameter of acervuli varied among the samples and it ranged from 100-180µm (Table 1). The spherical shaped acervuli were comparatively smaller in size ranging from 100-140µm while the hemispherical ones were larger (155-180µm). The average diameter of spherical, hemispherical and irregular shaped acervuli was 122µm, 171µm and 139µm, respectively. The fungus *C. capsici* produced profuse setae associated with the acervuli on PDA and the length of setae among the observed samples did not differ significantly. The setae grown in association with spherical, hemispherical and irregular acervuli varied in length ranging from 92-103µm, 93-110µm, and 96-104µm, respectively (Table 1). Minor variation was observed among the setae with respect to average length that ranged from 100µm to 104µm in spherical, hemispherical and irregular acervuli. Numerous conidia were developed within all the three different shaped acervuli of *C. capsici* grown on PDA and their length varied from 16µm to 25µm (Table 1). The conidia grown within the spherical, hemispherical and irregular acervuli did not differ sharply with respect to average size and was measured as 21µm, 20µm, and 21µm, respectively.

Source of inoculum: Apparently fruit rot infected portion of chilli was identified as a thick and dark black outline encompassing a gray to black or straw coloured area. Red coloured highly ripen fruits turned into straw coloured rotten fruit due to this disease. Numerous acervuli (fruiting body) of the fungus were scattered on the infected area (Fig. 1). Acervuli and stroma on the fruit were spherical to irregular in shape. Profusely developed septate, dark brown setae were present and scattered over the acervuli (Fig. 2). Aseptate and unbranched conidiophores bearing curved, unicellular narrow ends conidia, produced within acervuli, are the major source of inoculum for *C. capsici* (Fig. 2 & 5). These findings are supported by the reports of other researchers (Rangaswami 1996 and Singh 2004).

Deposition of propagules: The most essential feature of successful pathogenesis is the attachment of disperse propagules to the host surfaces. SEM observation indicated that conidia of 20µm size could easily adhere intimately on the 50µm curvature of cuticular layer (Fig. 3 & 4). It is seemed to the important step for successful pathogenesis resulting rapid infection the pathogen. Such observation was also supported by the reports of many investigators (Hamer *et al.* 1988; Agrios 2005).

Infection of host surface: Germination of conidia on healthy surface occurred after 24 hours of incubation. Conidia germinated to produce germ tube and ultimately formed sub-globosely appressorium that was the essential organ for initiating infection (Fig. 3). The morphological characteristics of these appressoria were similar to that of the genus *Colletotrichum.* The research reports on appressoria of *Colletotrichum* produced by other investigators indicated that the appressoria could be globose and sub-globose with or without lobes (Bailey and Jeger, 1992; Sutton 1968 and Lennĕ 1978).

Colonization: Light microscopic view of the infected host tissues revealed that the host cell walls were extensively degraded; cells become discoloured or dead and produced hyphal stroma (Fig. 5 & 6). Disrupted epidermal layers with cuticle found on the top of setae that resembled pushing out from inside (Fig. 2 & 6). These findings agreed with the report of Baily and Jeger (1992) who opined that setae with acervuli gave mechanical pressure from inside to rapture the epidermal wall and to tear the cuticle.

Thus light microscopy and scanning electron microscopy on fruit rot of chilli caused by *Colletotrichum capsici* indicated that after inoculation, conidia were germinated into germ tube and formed appressoria which were the most essential organ of the pathogen for initiating infection. Red coloured ripen fruits turned into straw coloured rotten fruit due to this disease. Infected portion of fruit become gray to black or straw coloured area with thick and dark black outline. Protruding fruiting bodies of the pathogen caused disruption of epidermal layers of host tissues and culminated to identifying acervuli and setae production on the chilli fruits.

Table 1. Morphomatrix of acervuli, setae and conidia of *Colletotrichum capsici* a fruit rot pathogen of chilli grown on potato dextrose agar medium measured under scanning electron microscope

Samples Observed	Shape of acervuli	Diameter of acervuli (µm)	Length of setae (µm)	Length of conidia (µm)
Sample # 1	Spherical	100	103	25
Sample # 2	Spherical	125	100	19
Sample # 3	Spherical	140	98	18
	Mean	122	100	21
Sample # 4	Hemispherical	155	105	16
Sample # 5	Hemispherical	170	93	20
Sample # 6	Hemispherical	175	106	23
Sample # 7	Hemispherical	177	110	18
Sample # 8	Hemispherical	180	107	24
	Mean	171	104	20
Sample # 9	Irregular shape	137	104	20
Sample # 10	Irregular shape	145	96	21
	Mean	139	100	21

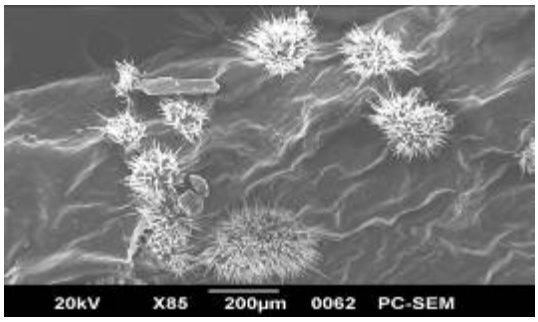

Fig. 1. Wrinkled surface of infected chilli showing acervuli with setae (SEM)

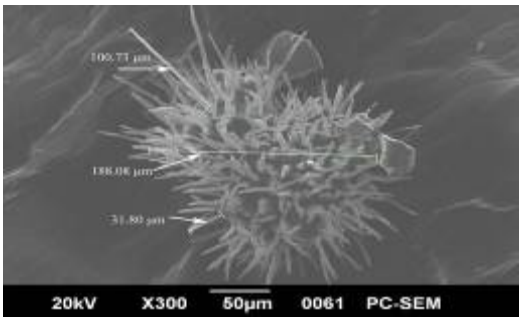

Fig. 2. Typical acervullus with thorn like setae pushing epidermal layer (SEM)

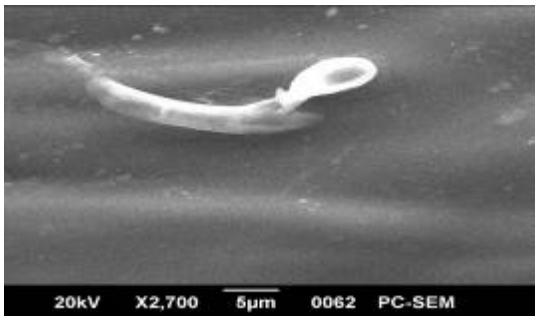

Fig. 3. Germination of adhered curved conidia of *C. capsici* showing narrow germ tube with lobed appressorium (SEM)

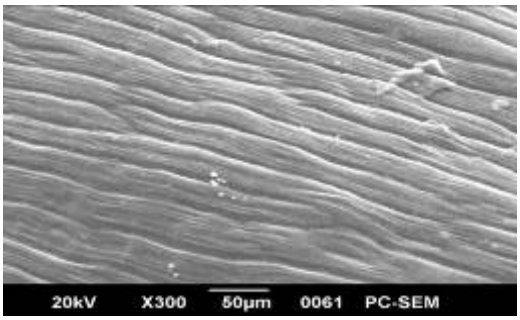

Fig. 4. Curvature surface view of healthy chilli (SEM)

Fig. 5. Acervuli on infected host tissue and conidia (LM)

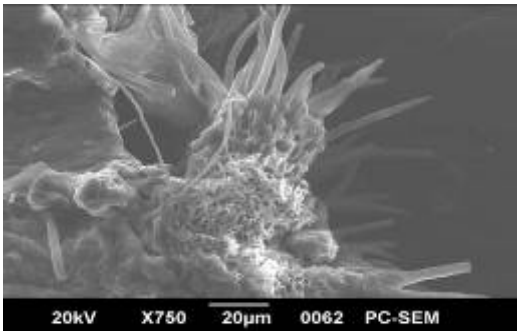

Fig. 6. Cross section of acervullus with rotten mass of host tissue (SEM)

CONCLUSION

The light microscopy and scanning electron microscopy in the present study has helped in achieving a clear picture of rapid pathogenesis of *Colletotrichum capsici* causing fruit rot of a susceptible chilli variety and also revealed the mechanism of obstructing pathogenic advancement.

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