Ultrastructure of conidium ontogeny in *Colletotrichum capsici*

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**ABSTRACT:** The conidia and conidiogenous cells of *C. capsici* the incitant of fruit rot and die back of chillies were studied for their ultrastructural and developmental features employing scanning (SEM) and transmission electron microscopy (TEM). The conidiogenous cells of the fungus resembled phialides. Formation of primary conidium is holoblastic while during secondary conidium development the outer layer of phialide wall ruptures above the basal septum of primary conidium leaving a collarette, showing enteroblastic conidiogenesis. The septum at the base of each conidium is complete and Woronin bodies are absent near the septum.

**Key words:** *Colletotrichum capsici*, ultrastructure, conidium ontogeny, Woronin body

Chilli (*Capsicum annuum* L.) crop has been reported to suffer due to the fungus *Colletotrichum capsici* (Syd.) Butler and Bisby causing ripe fruit rot and dieback symptoms. The pathogen is prevalent in most of the chilli growing areas of India (Rai and Chouhan, 1966; Bansal and Grover, 1969; Rout and Rath, 1972).

In Deuteromycetes taxonomy recently emphasis is laid on the mode of conidiogenesis. Cole and Samson (1979) described revised concept of phialidic development in some genera of Hyphomycetes and presented the major differences between phialidic and annellidic development on the basis of ultrastructural studies. Comparatively little attention has been paid on ultrastructural studies in genera of Coelomycetes (Sutton and Sandhu, 1969; Griffith and Campbell, 1972; Jones, 1976; Purohit and Chawla, 1997; Singh *et al.* 1997; Verkley, 1998 a and 1998 b). There are many gaps in our knowledge on the ultrastructural and developmental features of conidia and conidiogenous cells of *C. capsici*. Therefore, scanning and transmission electron microscopes were used to reveal the ultrastructural aspects of phialidic mode of conidiogenesis in *C. capsici*.

**MATERIALS AND METHODS**

Diseased samples were collected from the fields of chilli growing areas of Jodhpur and surrounding villages. The fungus was isolated and cultured on potato dextrose agar medium (PDA) at 28±2°C. The colonies of *C. capsici* developed within 2-3 days and sporulated within 7 days.

**Preparation of specimens for SEM**

Acervuli and conidia from sporulating culture of *C. capsici* were first fixed overnight in 2.5% glutaraldehyde. These were washed two times in 1% phosphate buffer (pH-7.2) for 1 hr and dehydrated in an acetone series of 50, 70, 80, 90 and 95% for 1/2 hr each followed by a final change in 100% copper sulphate. The samples were critical point dried in liquid CO₂ and mounted on specimen stubs and coated with gold. The material was examined under Philips SEM-513 B and LEO 4355 VP Stereoscan microscope.

**Preparation of specimen for TEM**

The sporulating culture was pre fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer for 2hr. These were then again washed in 0.1 M phosphate buffer for 1hr. These samples were dehydrated in 30, 50, 70, 80 and 90% acetone series for ½ hr each, followed by two changes in dry acetone and finally cleaned with toluene. Samples were immersed first in a mixture of araldite and toluene at room temperature and finally in araldite mixture at 50°C for 2 hr. The specimens were placed over night in the block of araldite mixture at 60°C in a polymerization oven. Semithin sections of samples (1u) were cut and stained with 1% toluidene blue in 0.1% borax and observed under light microscope for selecting the proper region. Subsequently ultra thin sections of the samples were cut with a glass knife on an ultratome, mounted on copper grids and post stained with saturated solution of 50% uranyl acetate and lead citrate for 10 minutes each. The specimen-mounted grids were dried and viewed under Philips C M 10 TEM.
RESULTS

In culture, \textit{C. capsici} develops a fruiting structure (acervulus) which consists of conidiogenous cells and setae (Fig. 1). The primary conidium arises as a protrusion of apex of the conidiogenous cell and develops holoblastically. This conidium initial grows, elongates and shows thickening of its vertical walls.

The developing primary conidium enlarges, and as the conidium matures, cell organelles migrate into it and a delimiting septum is formed. Neither Woronin bodies nor septal pores have been seen in association with delimiting septa. (Fig. 2, 3 & 4). The mature conidium reveals the presence of vesicles, endoplasmic reticulum, mitochondria and two big guttules within the cytoplasm (Fig. 7). The conidium secedes by a circumscissile break of wall material at the level above the basal septum leaving a collarette (Fig. 6). The secondary conidia developed enteroblastically from the conidiogenous cell (Fig. 6). Successive conidia are produced in a similar way from a fixed conidiogenous locus within the conidiogenous cell apex in a basipetal order. The wall of mature conidia consists of an outer electron dense layer and an inner electron transparent layer (Fig. 5). Thus \textit{C. capsici} is characterized by the phialidic mode of conidiogenesis.

![Fig. 1. Conidioma of \textit{C. capsici} (SEM micrograph x 505)](image)

![Fig. 2. Conidiogenous cell with developing holoblastic primary conidium (SEM micrograph x 2500)](image)

![Fig. 3. Conidiogenous cell with developing holoblastic primary conidium (TEM micrograph x 2050)](image)

![Fig. 4. Conidiogenous cell with holoblastic mature primary conidium (TEM micrograph x 2900)](image)
DISCUSSION

Cole and Samson (1979) and Cole (1981) on the basis of ultrastructural studies reported that the mode of formation of the first conidium in phialides and annellides is holoblastic involving essentially the same pattern. Present ultrastructural studies of conidiogenesis in *C. capsici* explicitly revealed that the first conidium in a phialide is formed holoblastically. The mode of development of secondary conidia of this species was clearly enteroblastic.

The relative positions of the circumscissile tear in the wall and the basal septum of the primary conidium have important implications in the taxonomy of both Hyphomycetes and Coelomycetes. Cole and Samson (1979) distinguished between annellidic and phialidic conidiogenesis on the basis of their ultrastructure observations of some Hyphomycetes and described that in the former the outer annellide wall ruptures at the base of the first formed conidium, adjacent to the double layered septum leaving an annellation while the outer wall of the phialide may rupture at any point above the basal septum, leaving a collarette. In the present fungus also the circumscissile tear in the wall of phialide occurs at a higher level than the septum. Therefore this species under study is characterized by a phialidic mode of conidial development.

The absence of Woronin bodies with the septa is considered to be typical characteristic feature of phialidic conidiogenesis (Carrol and Carrol, 1974; Jones, 1976; Cole and Samson, 1979), although exceptions to this feature are also reported (Khan and Aldrich, 1973). The septum at the base of each conidium in *C. capsici* was complete and Woronin bodies were also absent showing an important feature of phialidic development.

The number of conidia developed through each successively formed collarette is also considered as a characteristic feature in proliferating phialides. Sutton (1980) and NagRaj (1981) are of the opinion that since

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**Fig. 5.** Phialides with mature conidia (TEM micrograph × 2900)

**Fig. 6.** Conidiogenous cell with developing enteroblastic secondary conidium (SEM micrograph × 2500)

**Fig. 7.** Conidium of *C. capsici* (TEM micrograph × 2900)

ACV=Acervulus, ST=Setae, CC=Conidiogenous cell, PC=primary conidium, SC=Secondary conidium, COL=Collarette
the conidiomata of Coelomycetes are closed structures and conidiogenous cells are much smaller, therefore, it is difficult to interpret. In the present study neither striations in collarettes nor detectable elongation of the phialides were observed.

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REFERENCES


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