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Tsuno, Kazunori
Laboratory of Plant Pathology, Faculty of Agriculture, Kyusyu University

Mian, Ismail Hossain
Department of Plant Pathology, Institute of Postgraduate Studies in Agriculture (IPSA)

Bhuiyan, Khorshed Alam
Department of Plant Pathology, Institute of Postgraduate Studies in Agriculture (IPSA)

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Ultrastructures of Sclerotia of *Sclerotium rolfsii* during Their Formation and Germination

Kazunori Tsuno, Ismail Hossain Mian* and Khorshed Alam Bhuiyan*

Laboratory of Plant Pathology, Faculty of Agriculture, Kyusyu University, Fukuoka, Japan

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The morphogenesis and germination of sclerotia of *Sclerotium rolfsii* were studied comprehensively using a scanning electron microscope. It was found that the development of sclerotia is strand type and their germination is mycelogenic. Internal mass of a matured sclerotia is differentiated into a thin cuticle, a fairly thicker rind consisted of considerable densely arranged hyphae forming pseudoparenchymatous tissues having thick walled cells and a medulla consisted of loosely arranged ordinary filamentous hyphae forming prosenchymatous tissue. It was also observed that sclerotial germination may initiate from any location of surface, cuticle, rind, cortex, medulla or intermedullary layer by resuming growth of the hyphae which form the respective structures.

INTRODUCTION

Sclerotium rolfsii (Lib.) de Bary is a pathogen having wide host range causing root and foot rot, collar rot and wilt of many crops throughout Bangladesh (Talukdar, 1974 ; Ahmed and Hossain, 1985). The fungus produces hard mutlicellular resting bodies, known as sclerotia by which it survives in soil for a considerable period in absence of hosts. The structure may remain dormant for long time and germinate on the return of suitable conditions (Henis. 1979). Upon germination sclerotia produce vegetative mycelia (Coley-Smith, 1979). The morphogenesis of sclerotia in six species of fungi were studied using light microscope and three distinct types of sclerotial formation, viz. loose, terminal and mycelial strand types have been recognized. Among them the strand type of sclerotial development has been observed in S. *rolfsii* (Townsend and Willetts, 1954). It was also shown that a mature sclerotia of the fungus is differentiated into four distinct layers namely skin or cuticle, rind, cortex and medulla using light microscope (Townsend and Willetts, 1954) and phase contrast microscope (Chet *et al., 1965).*

The present paper will report the results of comprehensive studies on the morphogenesis and germination of S. *rolfsii* using scanning electron microscope.

MATERIALS AND METHODS

The isolate of S. *rolfsii* used for the study was obtained from Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh.

*Department of Plant Pathology, Institute of Postgraduate Studies in Agriculture (IPSA), Salna, Gazipur, Bangladesh.

It was isolated from root tissues of chick pea (*Cicer arietenum*) showing foot and root rot symptoms. To study the morphological changes during the process of sclerotial formation, specimens were collected from a fresh culture at different stages of development. Petri dishes (90 mm in diameter) containing 10 ml of potato dextrose agar (PDA) were inoculated in the center with agar discs covered with fungal mycelium, which had been cut from the edge of a 5 day-old colony with a cork borer having 5 mm diameter. Five pieces of cellulose membrane (10 mm × 10 mm) were placed surrounding the inoculum close to edge of Petri dish keeping equal distance among themselves and incubated at 25C. Specimens were collected by removing the membrane at the stages of formation of mycelial strands, sclerotial initials, cottony white mycelial aggregations, young sclerotia recognized as cottony white spherical bodies, immature sclerotia as whitish brown bodies and mature sclerotia as dark brown bodies. The above structures developed on the pieces of cellulose membrane were collected along with the membranes and prepared for scanning electron microscope (SEM) observation. They were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 4 hr at room temperature followed by washing with the same buffer for 3 times, postfixed in 2% osmium tetroxide in the buffer for 2 hr at 25C. Fixed specimens were dehydrated in five graded ethanol series viz. 50, 70, 90, 99.5 and 100% for 5, 10, 20, 20 and 60 min, respectively. Dehydrated specimens were air-dried and mounted on SEM copper stubs with double sided adhesive tape, coated with gold (ca. 230 Å) in a JFC-1100 Ion Sputter and observed under a JEM T-220 scanning electron microscope at 15 KV accelerating voltage. To observe the organization of internal tissues, fixed and dried specimens of matured sclerotia were cut into two halves with a single edge razor blade. The pieces were mounted on the SEM stub keeping the cut surface up, coated with gold and observed as mentioned above.

For observation of structural changes during germination, 30 matured sclerotia were selected and 10 of them were cut into two halves with the razor blade. Whole sclerotia as well as their halves were placed on cellulose membrane (10 mm X 10 mm) sheeted on PDA in Petri dishes. Each Petri dish received 5 pieces of cellulose membrane and a whole or a piece of sclerotium was placed at the center of the memrane. During placement of sclerotial pieces their cut surfaces were kept upwards. They were incubated at room temperature for 72 hr and germinating sclerotia or their pieces were observed with the SEM after fixation, drying and coating with gold ions following the method described above.

RESULTS AND DISCUSSIONS

In the early stage of sclerotial initiation, numerous mycelial strands were observed in the older regions of 72 hr old culture of *S. rolfsii*. Hyphal branches were found to be originated from the mycelial strands. Beginning of fusion of the branches and the mycelial strands were also observed (Fig. 1A). The sclerotial initials were produced from the intersections of the mycelial strands through multiple branching and interweaving of the hyphal branches (Fig. 1B). The initials were found to be composed of numerous hyphae laying more or less parallel with each other. Some of the hyphal branches grew outward from the strand. Branching of hyphae continued until the branches fused with other branches (Fig. 1C). The hyphae in the central region of

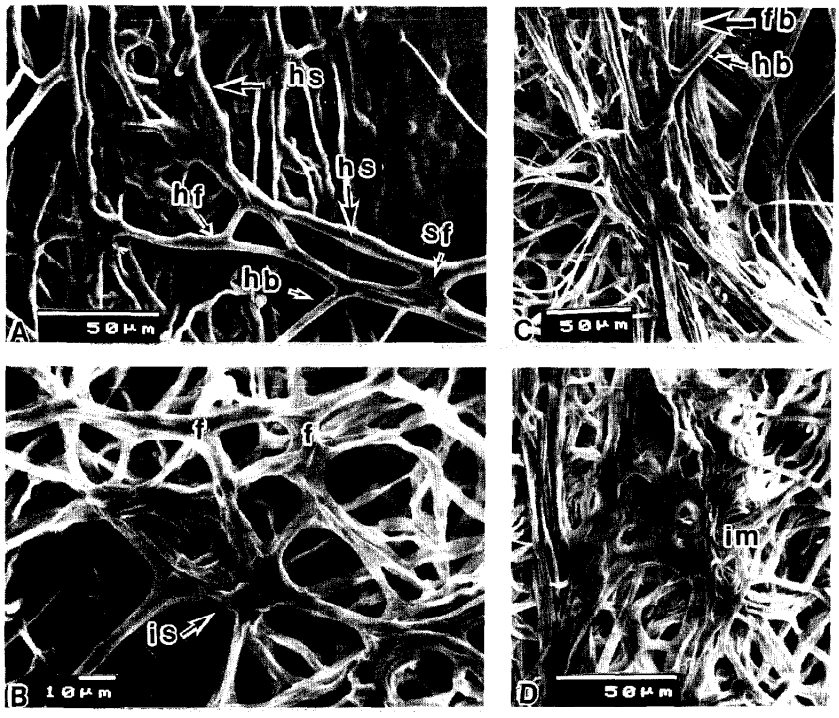

Fig. 1. SEM micrographs showing early stages in morphogenesis of sclerotia in *Sclerotium rolfsii*.

A. Mycelial stands showing hyphal branches and their fusion : (hb) Hyphal branching from the strands, (hf) Hyphal fusion, (hs) Hyphal strands, (sf) Fusion between hyphal strands.

B. Multiple branching of hyphae originated from the intersection of hyphal stands and fusion of the branches : (f) Fusion of hyphal branches, (is) intersection of hyphal strands.

C. A sclerotial initial composed of fused branches of hyphal strands lying parallel with each other : (fb) Fusion of hyphal branches lying parallel with each other.

D. A sclerotial initial composed of profusely branched but sparsely interwoved and fused hyphal branches : (im) Loosely interweaved mycelia.

sclerotial initial were profusely branched but loosely interweaved and some of them fused along their length (Fig. 1D).

On the later stage of development, sclerotial initials transformed into young sclerotia which were recognized in the mycelial system as white cottony masses composed of interweaving hyphal network (Fig. 2A). Within 24 hr the young sclerotia became spherical bodies having active hyphae on their surface. The hyphae were found to continue their growth projecting their tips outwards (Fig. 2A, B).

Immature sclerotia were recognized as brownish white spherical bodies. The hyphae on their surface were discontinued from the vegetative hyphae. Growth of the hyphae ceased but individuality of their tips was still recognizable (Fig. 2C, D). The

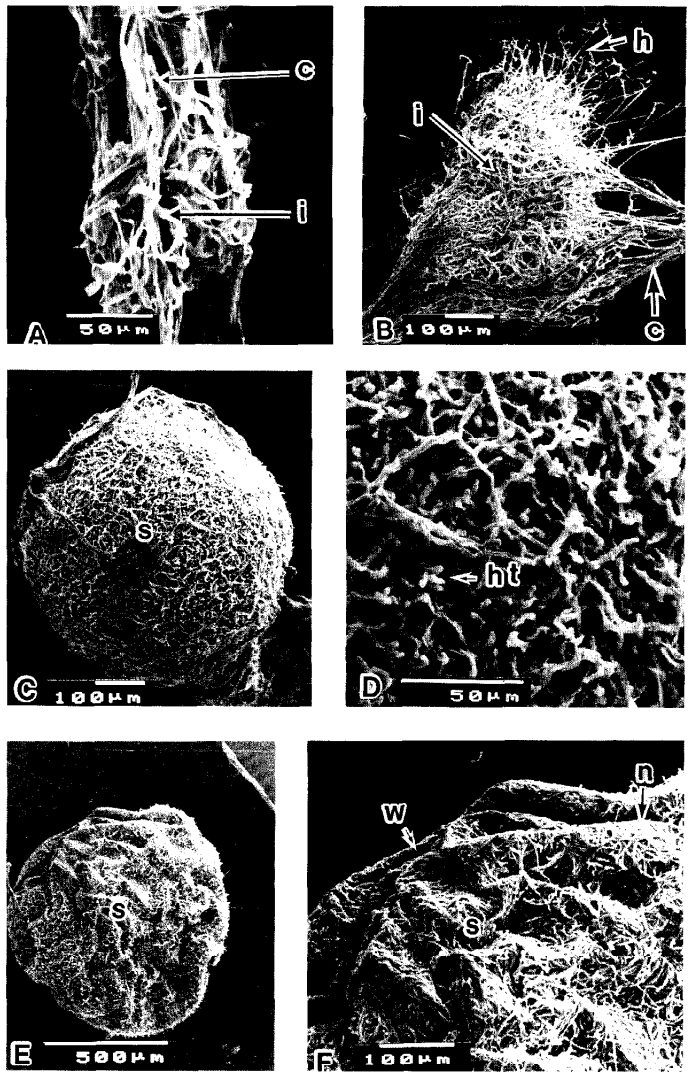

Fig. 2. SEM micrographs showing later stages in morphogenesis of sclerotia in *Sclerotium rolfsii.*

A. A young sclerotium composed of losely interweaved hyphal branches and connected with vegetative mycelium: (c) Connecting hyphae, (i) loosely interweaved hyphae.

B. An immature sclerotium composed of profusedly branched but sparsely interweaved hyphal branches (i) having active peripheral hyphae projecting their tips outwards (h) and connected with vegetative mycelium with hyphal branches (c).

C. A premature sclerotium having surface composed of active hyphae with free tips (s).

D. An enlarged view of the surface of the immature sclerotium showing active hyphal tips (ht).

premature sclerotia were observed as light brown spherical bodies with irregularly wavy surface. Growth of the hyphae on the surface ceased completely and coalesced considerably. Individuality of the hyphal tips were lost (Fig. 2D, E).

Mature sclerotia were dark brown spherical bodies with a smooth but uneven surface consisted of completely fused hyphal network (Fig. 3A, B). Observation of the transverse sections of matured sclerotia revealed that there are four recognizable zones as reported by investigators (Townsend and Willetts, 1954 ; Chet et al., 1965). The outermost zone or cuticle was a thin layer composed of completely fused hyphae. The next zone was thicker than cuticle, and composed of compactly interweaved hyphae forming pseudoparenchymatous tissues having thick walled cells. This zone is

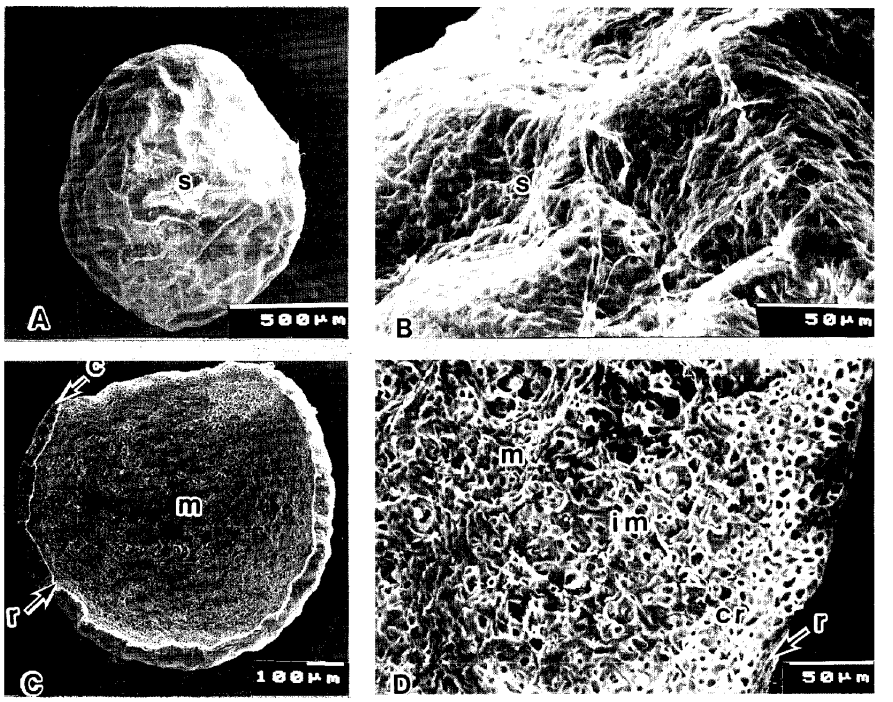

Fig. 3. SEM micrographs of mature sclerotia of *Sclerotium rolfsii* showing surface and internal masses.

A. A mature sclerotium with smooth but uneven surface (s) consisted complately fused mycelia.

B. An enlarge view of the mature sclerotium showing its surface (s) consisted of complately fused mycelia.

C. A transverse section of a mature sclerotium showing internal masses : (c) Cuticle, (m) medulla.

D. An enlarge view of the transverse section showing different zones in internal masses. (cr) cortex, (im) intermedullary layer, (m) medulla, (r) rind.

E. A premature sclerotium having surface consisted of hyphal network (s).

F. An enlarge view of the surface of premature sclerotium consisted of hyphal network : (n) Hyphal network, (s) surface, (w) wavy depression.

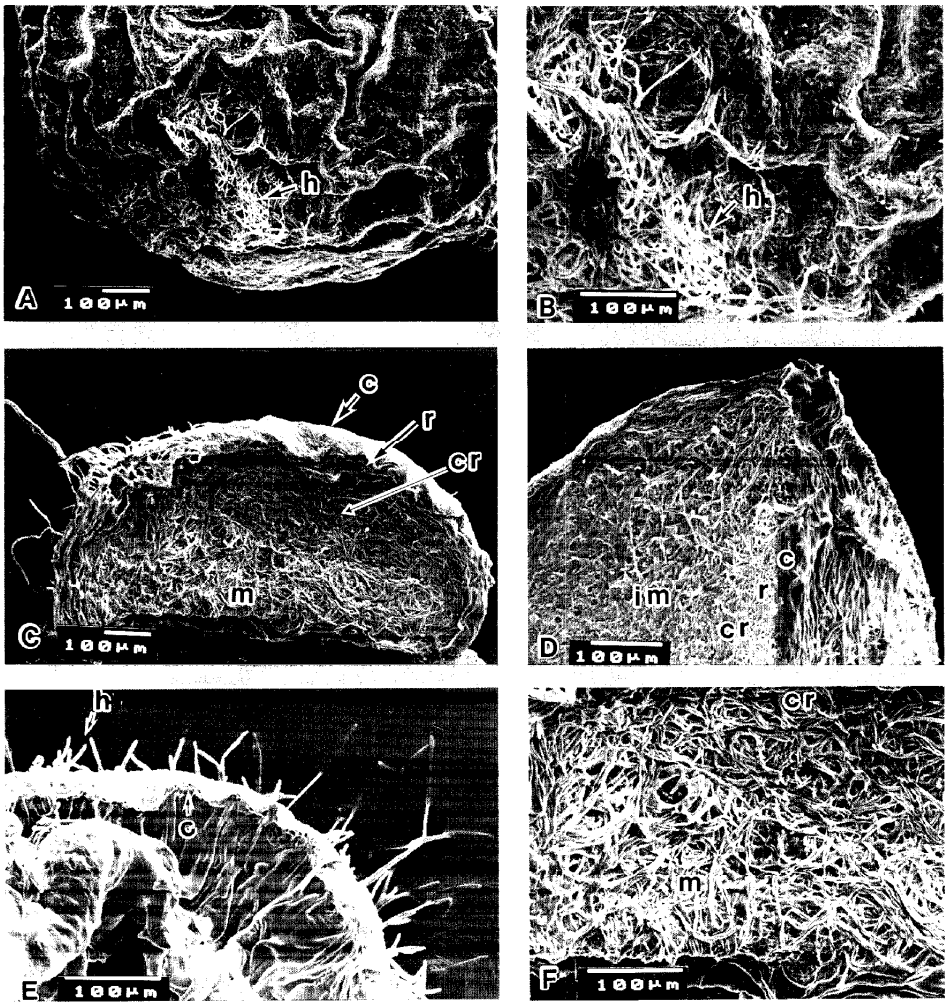

Fig. 4. SEM microgarphs showing germination of sclerotia of *Sclerotium rolfsii.*
A. Initiation of hyphae (h) from the sclerotial surface during germination.
B. An enlarge view of the germinating sclerotium showing resuming of hyphae (h) from the surface.
C. Germination of a cross section by resuming growth of hyphae from cuticle (c), rind (r), cortex (cr), and medulla (m).
D. Germination of a transverse section by resuming hyphal growth from cuticle (c), rind (r), cortex (cr), and intermedulary layers.
E. Initiation of germination from cuticle (c) producing outward growing hyphae (h).
F. An enlarge view of the germinating transverse section showing resuming of hyphal growth from cortex (cr), medulla (m) and intermedullary layer.

known as rind. The third zone or cortex was wider than rind having pseudoparenchymatous but its tissue consisted of thin walled cells with large intercellular spaces because hyphae of this zone were loosely arranged. The innermost zone, known as medulla, was found to occupy larger area of the internal mass of a sclerotium. It consisted of loosely interweaved, sparsely arranged and partially fused filamentous hyphae. The dimension of those hyphae appeared to be similar to vegetative hyphae. The tissue of this zone was found to be prosenchymatous with larger intercellular spaces (Fig. 3C, D).

It was recorded that germination of sclerotia was initiated by resuming the growth of hyphae. Hyphal growth was found to be resumed from any location of sclerotial surface, cuticle, rind, cortex, medulla and intermedullary layer (Fig. 4A, B, C, D, E, F). However, the location of germination needed to be direct contact with the culture medium.

The findings of the present study regarding differentiation and morphological changes occurred during initiation and development of sclerotia, and zonation of the internal mass of matured sclerotia of *S. rolfsii* are more or less in accordance with the findings of other investigators who studied the morphogenesis of sclerotia using light microscope or phase contrast microscope (Chet et **al.,** 1969 ; Townsend and Willetts, 1954). Though germination of sclerotium has been reported to be initiated from any location on the propagule (Coley-Smith, 1979) detailed information on the initiation of germination from the internal masses is lacking. The results presented in this paper are the detailed information on the morphogenesis and germination of sclerotia of S. *rolfsii* studied using SEM.

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