島根大農研報 (Bull. Fac. Agr. Shimane Univ.) 24 : 39−46, 1990

A Further Study on Ultrastructural Aspects of Photo-Induced Conidiogenesis and Dedifferentiation in *Alternaria solani*

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Alternaria solani における光誘導分生胞子形成および分生子梗脱分化の微細構造に関する続報

本田　雄一*・難波　信行**・野津　幹雄*

Ultrastructure of conidiophore has been studied in *Alternaria solani*, the pathogenic fungus of potato early blight, through the observation of the transverse sections as well as the longitudinal sections of the first cell of the fertile conidiophores and the blue light-irradiated, dedifferentiated conidiophores. The doughnut-shaped ETS at the apical dome and myelin figures closely associated with it were observed. The large vacuole developed in the middle part of the cell. Between the ETS and the vacuole many mitochondria mixed with the endoplasmic reticula converged. Nuclei were located below the large vacuole. Thus, the zonation of protoplasmic components was recognized in the first cell of the fertile conidiophore. In the later stage of the developmental stage a blue light irradiation induced to disperse the large vacuole and the conidiophore neither formed a conidium nor reverted to a vegetative hypha. The blue light-irradiated conidiophore at an early stage showed disorganization of the zonation of protoplasmic components, resulting in the vegetative regrowth of the conidiophore.

Introduction

In a previous paper we reported that in the fertile conidiophore of *Alternaria solani* (Ellis et G. Martin) Sorauer the electron transparent structure (ETS) developed at the apical dome, and a large vacuole in the first cell was presumed to provide turgor pressure for protrusion of the cytoplasm through the pore at the apex to form a conidium initial (Honda et al., 1987). These characteristic intracellular structures for the first cell of the fertile conidiophore were inhibited to develop by a blue light irradiation during the developmental stage of a conidiophore sensitive to the inhibitory irradiation, resulting in vegetative reversion of the conidiophore into an aerial hypha. These observations were made on longitudinal sections of the first

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cell of the conidiophore.

In this paper we describe the ultrastructural details of the first cell of the fertile conidiophore by observing transverse sections in comparison with the ultrastructure of the blue light-irradiated, dedifferentiated conidiophores.

Materials and Methods

Organisms: The isolate (A 18) of *Alternaria solani* for this study is the same one used in the previous report (Honda et al., 1987). The cultures of the isolate have been maintained on vegetable juice agar (VJA: Campbell's V-8 juice 200 ml, $CaCO_3$ 2 g, agar 17 g, distilled water 800 ml; pH 5.8) slants.

Culturing: Single spores from a newly sporulated colony were used as inocula to initiate cultures on VJA plates (10 ml/60 mm Pyrex Petri dish) and the colonies were incubated under darkness at 25 C for 4 days, at which time the colony was ca. 35 mm in diameter. Aerial mycelia of the colonies were then removed by a rubber spatula and the colonies were rinsed twice with sterilized, distilled water. Surfaces of these colonies were then dried under darkness at room temperature for 1 hr in a clean room. Colony margin was marked on the bottom of the Petri dish to locate the region to be sampled.

Irradiation: *Conidiophore induction*-The colonies were irradiated for 6 hr with near ultraviolet (NUV) radiation from two 10 W black light fluorescent lamps (FL 10 BLB, Matsushita Electric Co., Ltd., Osaka, Japan; emission range of 310-410 nm with the maximum at 360 nm) suspended 7 cm apart and 10 cm above the colonies to induce conidiophore formation. Irradiation was made through a Pyrex Petri dish lid which transmits radiation longer than 290 nm. Intensity of radiation was 370 $\mu W/cm^2$ at the colony surface. Intensities of radiation were measured by a thermopile having a quartz window (MIR-100 Q, Mitsubishi Yuka Co., Ltd., Yokkaichi, Japan) coupled with a digital multimeter (TR 6846, Advantest Co., Ltd., Tokyo, Japan) The colonies were transferred to darkness after 6 hr inductive NUV irradiation.

Dedifferentiation of conidiophores-In order to induce dedifferentiation of conidiophores, the colonies were irradiated for 2 hr with blue light after 6 or 10 hr of darkness following 6 hr inductive NUV irradiation. The blue light was obtained by filtering white light from three daylight fluorescent lamps (FL 10 D, Mitsubishi Electric Co.,

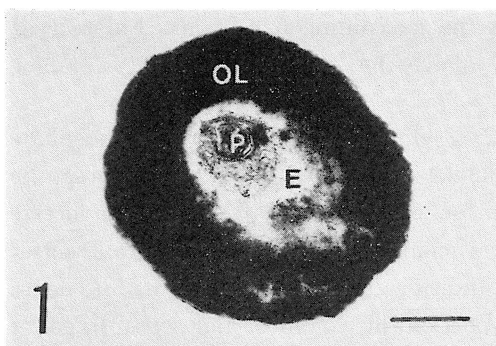
Fig. 1. Transverse section 0.5 μm from the tip of the fertile conidiophore immediately before the conidium formation. Osmiophilic outer layer (OL) of the cell wall of the apical dome is conspicuous. An apical pore (P) is detected at the center of the electron ransparent structure, ETS (E). Scale = 0.5 μm

Fig. 2. Transverse section 1 μm from the tip. The distinct ETS (E) surrounds the cytoplasm filled with apical vesicles (A). Some myelim figures (MF) associate with the ETS (E). Scale = 0.5 μm

Fig. 3. Transverse section 2-3 μm from the tip. Some round mitochondria (M) lie in the central zone of the cytoplasm. Myelin figures (MF) form a broad ring around this zone. Scale = 0.5 μm

Fig. 4. Transverse section 5-10 μm from the tip. Many mitochondria (M) are mixed with the endoplasmic reticula (ER). There is no vacuole in this zone. Inner layer (W) of cell wall is electron transparent. Scale = 0.5 μm

Fig. 5. Transverse section of the middle part of the first cell. A large vacuole (V) squeezes the cytoplasm involving mitochondria (M) to the cell wall. Scale = 0.5 μm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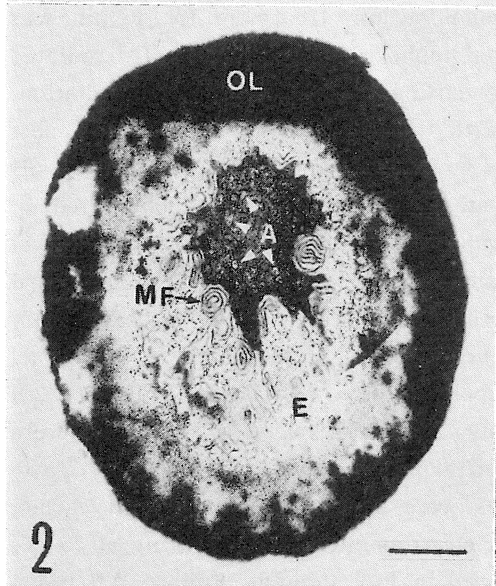
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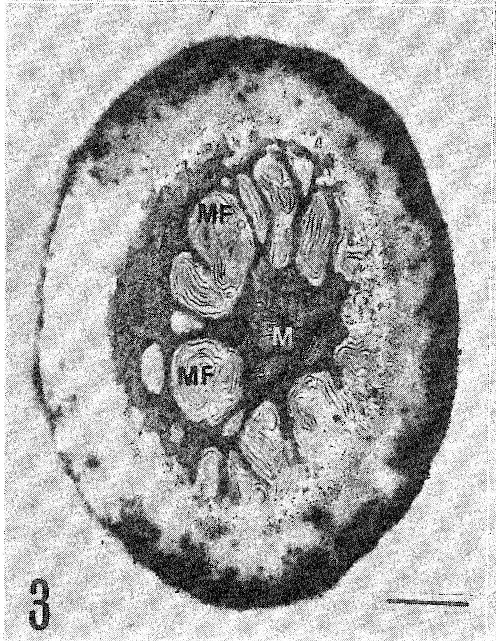
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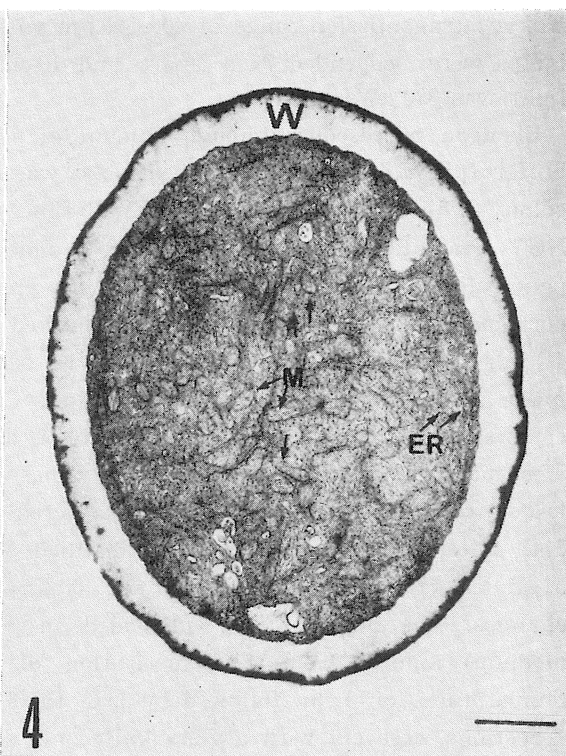
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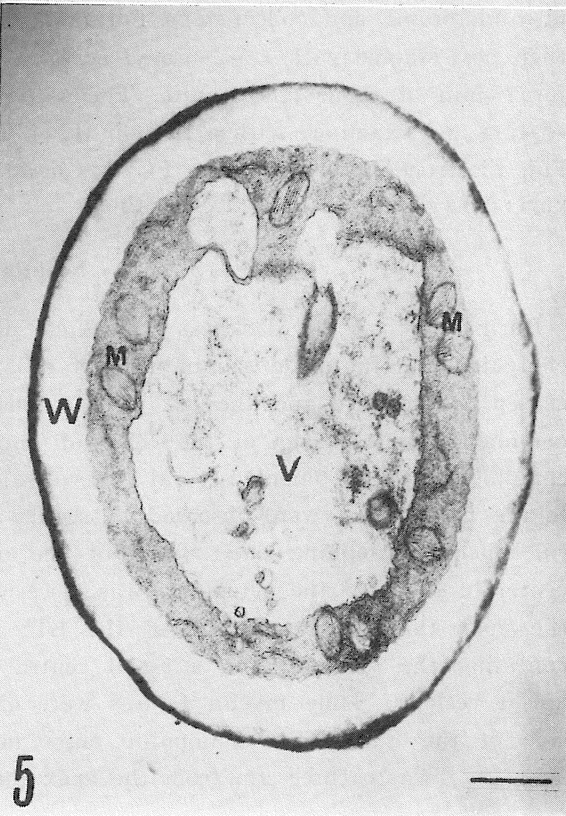
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Ltd., Tokyo, Japan) through a band pass filter (BPB-45, Fuji Photo Film Co., Ltd., Tokyo: transmission range of 400-500 nm with the maximum at 450 nm). Fluorescent lamps were suspended 7 cm apart and 10 cm above the colonies. Intensity of blue light was 320 μW/cm^2.

Electron microscopy: Enough amount of 6.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) with 0.1% Tween #20 was added to a Petri dish to immerse the colony which was incubated under darkness for 8 or 10 hr after 6 hr inductive NUV irradiation. Colonies were treated under vacuum for 15 min to facilitate better penetration of the fixative. After this pre-fixation chunks (1 X 5 mm) of dense conidiophores were taken from the marked portion of colonies and were fixed at 4C for 3 hr in the same fixative. The second vacuum treatment for 15 min was made at the beginning of 3 hr fixation. Conidiophores from blue light irradiated colonies were also fixed at the end of 2 hr darkness after blue light irradiation. The material was then washed three times with 0.1 M phosphate buffer, and post fixed for 3 hr with a phosphate-buffered 1% osmium tetroxide solution at pH 7.4. After three time rinses in distilled water, the material was dehydrated in a graded ethanol series followed by 100% propylene oxide for 1.5 hr with several changes. The material was embedded in Spurr's low-viscosity medium (standard medium) (Spurr, 1969). Polymerization of the resin was carried out at room temperature for 1 hr followed by 45 C for 6 hr, 60 C for 12 hr and 70 C for 24 hr.

Sections were cut with a glass knife or a sapphire knife on a Porter-Blum MT-2 B ultramicrotome and collected on Formvar-coated 100-mesh copper grids. They were then post-stained with 7.5% uranyl acetate for 30 min followed by 2.2% lead citrate for 15 min at room temperature. The sections were stabilized with a light carbon coating and examined with a Hitachi HU-12 A electron-microscope working at 75 kV. Fuji Electron-Microscopic Film FG was developed in Fuji Copinal. Fujibro WP FM 4 paper was developed in Fuji Microfine.

Results

During 6 to 8 hr darkness following inductive NUV irradiation conidiophores elongated and increased the number of cells. Then conidiophores with 3 to 4 cells ceased to elongate and the cell wall of the apical dome thickened, especially an osmiophilic outer layer of the cell wall increased its thickness. At this stage of conidiophore development, several transverse sections of the first cell near the apex to the middle part were observed. A section within 0.5 μm of the apex showed the thickened, osmiophilic outer layer of the cell wall lined with the ETS. At the center of the ETS the cytoplasm was discernible (Fig. 1). Approximately 1 μm from the apex the entire structure of the ETS appeared, lining the apical dome and confining the cytoplasm to a small central area. The cytoplasm was full of the apical vesicles. Some myelin figures were observed associating with the cytoplasm side of the ETS. The osmiophilic outer layer of the cell wall was conspicuous (Fig. 2). Two to three μm from the apex the cell wall was about 0.5 μm thick and

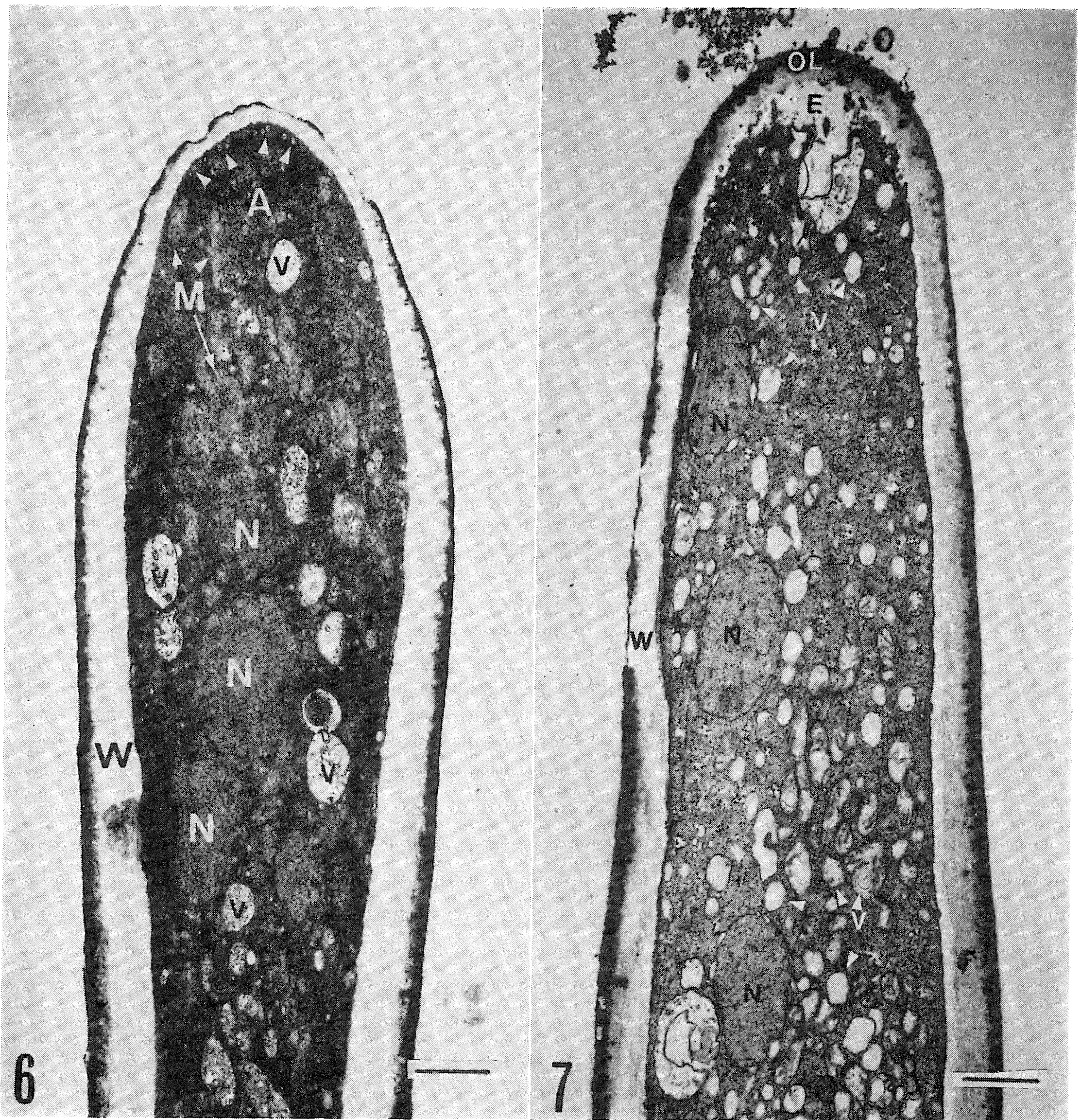

Fig. 6. Longitudinal section of the first cell of the conidiophore irradiated by blue light at early developmental stage before the formation of the ETS and the large vacuole. Apical vesicles (**A**) converge to the apex and the cell wall of the apical dome is not thick. Scale = 1 μm

Fig. 7. Longitudinal section of the first cell of the conidiophore irradiated by blue light at the later stage after the formation of the ETS and the large vacuole (**V**). The large vacuole is dispersed into many small vacuoles, while the ETS (**E**) and thick outer cell wall (**OL**) of the apical dome remain unchanged. Scale = 1 μm

the electron transparent inner layer comprised more than half of the cell wall (Fig. 3). The ETS disappeared and, instead, many myelin figures appeared surrounding the central part of the cytoplasm which was filled with mitochondria. A concentration of mitochondria was recognized at 5 to 10 μm from the apex of the fertile conidiophore and called a mitochondrial region (Fig. 4). The mitochondrial

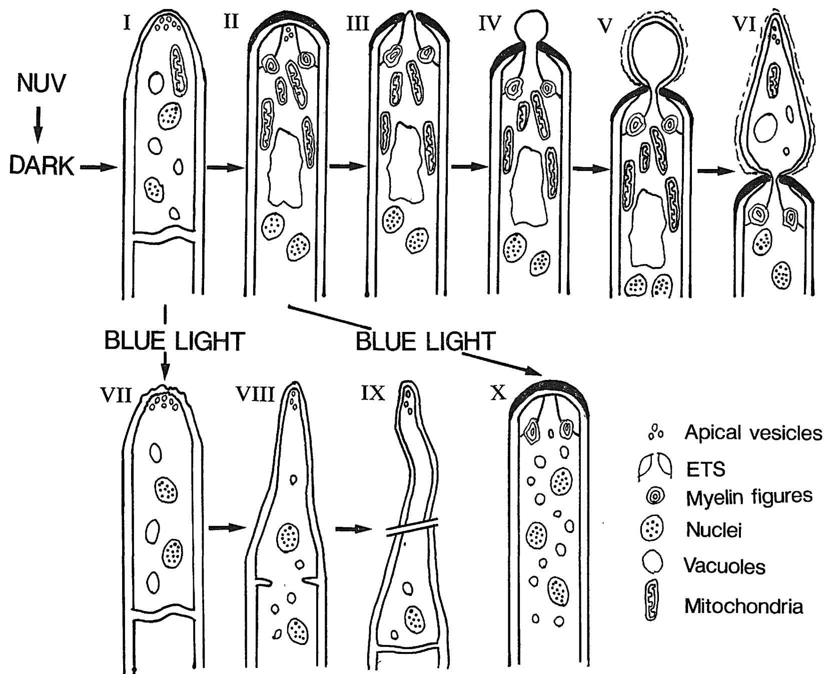

Fig. 8. Diagrammatic representation of conidiophore development and dedifferentiation induced by a blue light irradiation at two different stages. Without the blue light irradiation conidium formation proceeds through stage **I** to stage **VI** under dark. Dedifferentiation induced by the blue light irradiation at an early developmental stage of the conidiophore (**VII** to **IX**) and at a later developmental stage (**X**).

region was mixed with the endoplasmic reticula (ER). The osmiophilic outer layer decreased the thickness to one fifth of the cell wall. Many of the mitochondria had a round shape, indicative of the transverse section of the elongated, fusiform mitochondria.

In the middle part of the first cell of the fertile conidiophore a large vacuole developed and the cytoplasm was pushed aside to the cell wall (Fig. 5). Many mitochondria in the narrow cytoplasm showed a round shape, indicating the arrangement of the elongated, fusiform mitochondria parallel to the long axis of the cell around the well developed, large vacuole.

In contrast to the fertile conidiophores, blue light-irradiated conidiophores were inhibited to form conidia and showed vegetative reversion to aerial hyphae. After 6 hr darkness following inductive NUV irradiation, conidiophores without a thick cell wall of the apical dome and a large vacuole were exposed to a 2 hr blue light irradiation. After additional 2 hr darkness following the blue light irradiation apical vesicles converged to the apex of the conidiophore and cell wall of the apical dome decreased its thickness (Fig. 6). The thick osmiophilic outer layer of the apex did not develop at all. The large vacuole was also inhibited to develop. In the first cell of the blue light-irradiated conidiophore small vacuoles remained to distribute throughout the cell. The apex of the conidiophore became sharp, indicating the onset of

vegetative regrowth as an aerial hypha. When the conidiophores were irradiated by blue light after 10 hr darkness following the inductive NUV irradiation, conidiophores having a well developed large vacuole, the ETS and the thick osmiophilic outer layer of the cell wall at the apical dome were induced to disperse the large vacuole to many small vacuoles, while the ETS and the thick outer cell wall of the apical dome remained unchanged. Dispersion of the large vacuole resulted in inhibition of conidium formation (Fig. 7). At this developmental stage blue light did not affect the thick cell wall of the apical dome and the ETS inside the apex, resulting in standstill of conidiophore development (Fig. 8).

Discussion

Internal organization of the first cell of the fertile conidiophore observed by several sequential transverse sections is consistent with that observed by longitudinal sections. Characteristic features of the fertile conidiophore are the thick osmiophilic outer layer of the cell wall at the apical dome, the doughnut-shaped ETS with a central cavity filled with apical vesicles, myelin figures under the ETS, a distinct mitochondrial region in the subapical zone of the first cell, and a well-developed, large vacuole. The concentration of apical vesicles in the cytoplasm of the ETS central cavity in the first cell of the fertile conidiophore is consistent with the cytoplasm of the apical dome in a growing, vegetative hypha, which consists mainly of apical vesicles to the near or total exclusion of other organelles (Bartnicki-Garcia, 1973). In the vegetative hyphae the concentration of apical vesicles leads to establish a pattern of wall construction that will continuously transform the hemispherical surface of apical dome wall into a cylindrical surface. In contrast, the thick cell wall with a apical pore and a doughnut-shaped ETS lining the apical dome of the fertile conidiophore are presumed to confine the cytoplasm filled with apical vesicles to a small area of plasma membrane and to bring a change in growth pattern of the apical cell from cylindrical to spherical growth, thus results in formation of spherical conidium initial in the fertile conidiophore.

Sequential transverse sections of apical zone of the fertile conidiophores (Fig. 2, 3) show a close association of myelin figures with the ETS, suggesting that myelin figures merge with the plasma membrane of apical dome and form the ETS.

The subapical zone just below the ETS and myelin figures is characterized by a dense accumulation of protoplasmic components including mitochondria, ribosome, ER, vesicles, but it lacks vacuoles. The mitochondria dominate among the protoplasmic components and they have a round shape in transverse sections (Fig. 3, 4). The elongated, fusiform mitochondria were recognized in the subapical zone of the first cell of the fertile conidiophore in the previous study (Honda et al., 1987). The elongated mitochondria were reported to be characteristic for the tip of growing, vegetative hyphae of *Allomyces arbuscula* (Roos and Turian, 1977). Thus, the profile of protoplasmic components is almost the same with of a growing vegetative hypha

(Grove et al., 1970). In the fertile conidiophore, however, a large vacuole develops in the middle part of the cell. The appearance of a large vacuole is abrupt rather than a gradual transition from a mitochondrial region in the subapical zone to the zone of vacuolation. Vacuoles of a small size are not present in this zone and the bulk of the protoplast is comprised of a large vacuole, other intracellular components being squeezed between the cell wall and the vacuole. Two to four nuclei are located below the vacuole (Fig. 8).

In the developmental stage after formation of the ETS and the large vacuole, a blue light irradiation disperses the large vacuole, resulting in uniform distribution of many small vacuoles throughout the first cell of the conidiophore (Fig. 7,8). Concomitantly the accumulation of apical vesicles at the apex, the mitochondrial region and the nucleic zone disappear. Nuclei, mitochondria, small vacuoles, vesicles and other protoplast components are mixed with each other, thus resulting in disorganization of the zonation in the protoplasmic components, which is always recognized in the first cell of the fertile conidiophore as well as in the apical cell of a growing, vegetative hypha (Grove et al., 1970). The blue light-irradiated conidiophores with the disorganized zonation neither form conidia nor revert to the vegetative hyphae.

In the stage before the formation of the ETS and the large vacuole, the blue light irradiation inhibits the formation of these structures, and induces accumulation of apical vesicles at the apex (Fig. 6). The cell wall at the apex has a more uneven profile and the plasma membrane has also an irregular profile, the same characteristic with the growing apex of a vegetative hypha. The conversion of the protoplasmic organization leads to the vegetative regrowth of the conidiophore.

Acknowledgments

We wish to thank Mr. Terumasa Katakami for his technical assistance in printing the photographs.

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摘　　要

ジャガイモ夏疫病菌 (*Alternaria solani*) における分生子梗の微細構造について，分生胞子形成分生子梗および青色光照射による脱分化分生子梗の縦断及び横断切片を観察し検討した．ドーナツ形の電子透過構造物 (Electron transparent structure ＝ETS) が分生子梗先端半球の内側に形成され，これに密接に結合しているミエリン様体が観察された．大きな液胞が細胞の中央部に形成され，ETS と液胞の間には多数のミトコンドリアが小胞体と共に集中している部位があった．大きな液胞の下部には核が集積していた．分生胞子を形成する分生子梗の先端細胞にはこのように細胞内器官が層状に分布していた．分生子梗の生育後期における青色光照射は大きな液胞を分散させ，分生子梗は分生胞子を形成せず，脱分化もしなかった．一方，分生子梗の発育初期に青色光が照射されると，分生子梗先端細胞における細胞内器官の層状分布が乱れ，分生子梗は栄養菌糸に脱分化した．