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ULTRASTRUCTURAL ASPECTS DURING SPORANGIUM DIRECT GERMINATION IN *PERONOPHYTHORA LITCHII*[1,2]

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Abstract

Light and electron microscopy of two modes of sporangium direct germination in ***Peronophythora litchii***, with normal germ tube on V-8 juice agar and with thin germ tube on 2% water agar, were described. The flagella and cleavage system degenerated prior to normal germ tube formation. Subsequently, numerous dictyosome-derived vesicles moved toward the sporangial wall at the site where the nascent germ tube was deposited between the dissolved sporangial wall and the plasma membrane. As soon as perforating through the sporangial wall, the emerging normal germ tube started to extend and branch, and ultimately, it entered into the mycelial stage. Concurrently, in the sporangium part of the germling, the cytoplasmic changes inclined to those at the hyphal tip. However, some variances existed between these two modes of germination. Directly germinating sporangium from 2% water agar mostly had a single germ tube, thinner than the normal one, emerging frequently from the papilla. After the thin germ tube emergence, its growing rate was slower and no branching was observed. Further, the number of dictyosome was not as many as in the normal germling and its activity was lower.

Introduction

Direct germination of sporangia of *Peronophythora litchii* was reported to take

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[4] Abbreviations: CEV=central vacuole; CV=cleavage vesicle; D=dictyosome; dW=dissolved sporangial wall; ER=rough endoplasmic reticulum; F=flagellum; FIV=fibrillar vesicle; FPV=fingerprint vacuole; FV=flagellar vacuole; GT=germ tube; HW=hyphal wall; LB=lipid body; LM=light micrograph; M=mitochondria; Mb=microbody; N=nucleus; NP=nuclear pore; P=papilla; PM=plasma membrane; SW=sporangial wall; Te=transitional element of endoplasmic reticulum; tW=germ tube wall; V=cell wall vesicle; SEM=scanning electron micrograph; TEM=transmission electron micrograph.

place at higher temperature (28°C) on V-8 juice agar, while sporangia can differentiate and release zoospores by cold shock (12–16°C) in the presence of free water (Kao and Leu, 1980). Similar ways of germination were found in the genus *Phytophthora* (Hemmes and Hohl, 1969; Ribeiro, 1983). However, in *Peronophythora*, two modes of directly germinating sporangia, by normal germ tube on V-8 juice agar and by thin germ tube on 2% water agar, were found (Kao and Leu, 1980). The present study was aimed at the electron microscopy of these two modes of direct germination, in order to investigate their cytoplasmic events and to compare with those previously described in *Phytophthora* species (Hemmes and Hohl, 1969). According to Smith *et al.* (1976), the germination of nonmotile fungal spore was divided into three phases: 1. the ultrastructural changes within the spore before germ tube emergence; 2. the protrusion of the germ tube from the spore wall; 3. the elongation of the germ tube and the establishment of the hyphal tip. This provides a basis for comparative studies and description in direct germination of *Peronophythora* sporangia.

Materials and Methods

An isolate of *Peronophythora litchii*, PPCT P 120, originally isolated in 1977 at Plant Protection Center, Taiwan, was used. Cultures of the fungus were grown in light at 26°C on V-8 juice agar (10% V-8 juice agar, Campbell Corp., 0.2% $CaCO_3$, 2% agar) in 9 cm petri dishes. After 7–8 days of incubation the cultures were inversely printed on fresh V-8 juice agar plates. These re-inoculated plates were then placed in light at 26°C for 32 h. The cultures, now bearing abundant sporangia (Figs. 1, 2), were used to induce direct germination of sporangia. These cultures were inversely printed on V-8 juice agar or on 2% water agar, then all incubated in light at 28°C for 80 min and for 150 min, respectively. They were then fixed by adding 3% glutaraldehyde in 0.05 M sodium-cacodylate buffer at pH 7 for 0.5 h and were brushed lightly with a brush to obtain sporangia suspension. The suspension was centrifuged at 590 g for 10 min and the pellets were mixed with an equal volume of 3% molten agar. The resultant agar pellets were cut into small blocks and resuspended in fresh fixative for 1.5 h at room temperature. After being washed for 30 min in three changes of buffer at pH 7 for 1.5 h at room temperature, then rinsed for 30 min in three changes of distilled water and stained for 3 h in 0.5% aqueous uranyl acetate, the blocks were dehydrated in graded series of ethanol and embedded in Spurr's resin. Thin sections were poststained in lead citrate and examined under a Hitachi H-300 electron microscope using 75 Kv accelerating voltage.

For scanning electron microscopy, specimens of directly germinating sporangia were vapor-fixed for 6 h at room temperature with several drops of 2% phosphate-buffered osmic acid. The specimens were dehydrated in a graded series of ethanol,

and then immersed in absolute amyl acetate overnight. After being critical-point-dried, samples were gold-coated in an Akashi evaporator and photographed with a Hitachi S-410 scanning electron microscope.

For light microscopy, mature sporangia and directly germinating sporangia were observed and photographed with Zeiss photomicroscope.

Results

Ultrastructural Changes within the Sporangium before Germ Tube Emergenence

Cytoplasmic events in the mature sporangium of *Peronophythora litchii* had been described previously (Lin, 1980). When the mature sporangia were induced to germinate directly, the morphology of subcellular organelles gave rise to some changes. The large flagellar vacuole (Fig. 10) had been cleaved into several smaller vesicles which always contained multi-layered vesicular membranes (Fig. 12). These vesicular membranes were the traces of flagellar degeneration. The dictyosomes were often distributed in the periphery of the nucleus (Figs. 11, 12). These organelles did not produce cleavage vesicles as in the mature sporangium (Fig. 10), but secreted cell wall vesicles which often isotropically underlied the plasma membrane (Fig. 11), and similar to those described in indirectly germinating sporangium (Lin, 1980). Although no newly formed vesicular elements just below the sporangial wall completely around the protoplast was evidently observed as described in *Phytophthora parasitica* (Hemmes and Hohl, 1969), there was a thin wall-like layer that might be newly formed around the whole sporangial protoplast in the directly germinating sporangium with thin germ tube (Figs. 24, 25). The fingerprint vacuoles had not yet lost their structure, but appeared less clear (Figs. 11, 13). The osmophilic lipid body revealed electron-denser in staining, but more electron-transparent in the periphery (Fig. 11). The nucleus yet maintained its pyriform shape (Fig. 11). In contrast with the mature sporangia, the narrow portion of the nucleus in the induced sporangia was not always toward the sporangial wall (Fig. 11) and several layers of endoplasmic reticulum were not observed around the nucleus (Figs. 11, 12). In addition, the outer membrane of nuclear envelope was sometimes attached by aggregated ribosomes, concurrently, the inner membrane was not edged by the elctron-dense nucleoplasm (Figs. 11, 12). The number of nuclear pore was not as many as in the mature sporangium (Fig. 10). The morphology of the nucleus was very similar to that found at the hyphal tip. The plasma membrane of the induced sporangium was acutely angled, forming a rugose, folding appearance (Figs. 11, 12).

Protrusion of the Germ Tube from the Sporangial Wall

Just prior to the germ tube formation, numerous cell wall vesicles appeared to

be aggregated near the *trans* side of the cytoplasm (Fig. 15) as those found in germ tube tip (Figs. 18, 19) and in the hyphal tip (Fig. 30). Obviously, a spacious accumulation of these vesicles occurred in the periphery of the sporangial cytoplasm (Fig. 15) near the site where the nascent germ tube was later initiated. These vesicles were gradually transported to a restricted area of the surface of the round cell where germ tube would be formed and emerge (Fig. 16). The inner side of the original sporangial wall over this restricted area was transformed from electron-translucent into electron-dense granular structure (Fig. 16) which developed upward along the nascent germ tube perforating through the original sporangial wall. Subsequently, in this area, gathering more cell wall vesicles, the nascent germ tube wall was newly formed between the dissolved sporangial wall and the plasma membrane (Fig. 17). In the same time, this newly formed germ tube was slightly tapering and, shortly afterward, broke through the sporangial wall (Fig. 18). This incipient germ tube was filled with numerous cell wall vesicles as in the hyphal tip (Fig. 30). As soon as perforation through the sporangial wall was done, the emerging germ tube was enlarged and, the mitochondria and endoplasmic reticulum were seen in the germ tube (Fig. 19). In the sporangium part of the germling, the shape of mitochondria changed from stumpy to elongated and narrow. The cleavage vesicles, the fingerprint vacuoles, the lipid bodies and the central vacuole were aggregated together and inclined to fuse one another (Figs. 20, 21).

In general, there were often several germ tubes emerging from random positions of the sporangium exposed to V-8 juice agar (Figs. 3, 4, 8, 9). However, a sporangium exposed to 2% water agar, a single germ tube was frequently protruding at the papilla region (Figs. 6, 7, 23). Although its germination process was similar to that in the normal germination, the cytoplasmic phases of sporangia revealed a little difference. There were nine sets of dictyosomes found in the normal germinating sporangium (Fig. 20), but only one set of dictyosome, which lacked the characteristic secretory vesicles and appeared reduced (Fig. 14), was observed in a germinating sporangium with thin germ tube (Fig. 21). The sporangial protoplast was separated from the sporangial wall (Figs. 22, 24, 25). Furthermore, the formerly formed thin layer wall, which was continuous with the newly formed germ tube wall at present, was slightly departed from the sporangial wall (Fig. 25). However, the germ tube was yet in close association with the germ tube cytoplasm (Figs. 22, 27).

Elongation of the Germ Tube and the Establishment of the Hyphal Tip

After emergence and enlargement, the germ tube started to elongate (Fig. 3), then branched (Fig. 4). Subsequently, it entered into the mycelial phase (Fig. 5). However, in 2%-water-agar induced sporangium, germ tube (Figs. 6, 7) did not branch and was narrow in width than that in normal induced sporangium (Fig. 4).

The cytoplasm of these germinating sporangia in mycelial phase increased in vacuolation (Fig. 28). The fingerprint vacuoles had completely lost their fingerprint structure and contacted with each other (Fig. 28). Later, the electron-transparent vacuoles appeared in the cytoplasm and gradually occupied the bulk of the cytoplasm (Fig. 29). In 2%-water-agar induced sporangium, its darker materials were seen transported to the germ tube area along the thin germ tube development (Figs. 6, 7). In normal induced sporangium, some darker materials were yet remained in the sporangium of the germling (Fig. 5).

While the thin germ tube was elongating, the sporangial wall near the germ tube was also extended and thinner than the original sporangial wall. Furthermore, a thin papilla layer was seen over the extended sporangial wall (Figs. 26, 27).

The cytoplasm of the hyphal tip developed from the germ tube was filled with abundant cell wall vesicles and a few ribosomes (Fig. 30). Behind this vesicular zone, elongated mitochondria, dictyosomes and segments of endoplasmic reticulum were found (Figs. 30, 31). The mitochondria were often seen near the haphal wall (Fig. 32). The hyphal wall was consisted of two layers, the amorphous outer layer and the electron-translucent fibrillar inner layer (Figs. 30, 32).

Discussion

Generally, the ultrastructural changes in directly germinating sporangium of *Peronophythora litchii* are similar to those described in *Phytophthora parasitica* (Hemmes and Hohl, 1969). The mature sporangium of *Peronophythora litchii* has directly developed to be able to process immediately the indirect germination in free water (Lin, 1680), therefore, the cytoplasm of the mature sporangium is not in resting phase resembling to that in the dormant spore. Thus, the flagellar degeneration and the cease of the cleavage vesicles secretion are the major cytoplasmic events during the period prior to the germ tube formation. On the other hand, this initially induced sporangium is in the absence of cell expansion or wall thickening before forming the germ tube, which is generally occurred in the germination of the nonmotile spore (Smith *et al.*, 1976). Nevertheless, there is a thin layer structure formed around the sporangial protoplast before the germ tube formation. After the germ tube emergence, the induced sporangium inclines to changing its cytoplasm into that of the hypha and its dictyosomes proliferate numerous cell wall vesicles for the establishment of the initial germ tube wall and subsequent germ tube formation. In *Phytophthora parasitica*, Hemmes and Hohl (1969) stated that direct germination might be regarded as an intrasporangial encystation of partially formed zoospore. We agree to that and further indicate that cytoplasmic changes in direct germination of sporangium are very similar to those in the cyst germination.

Hegnauer and Hohl (1973) described that the cyst germ tube wall was not a simple extension of the cyst wall but a new structural entity separated from the cyst wall by thin line of demarcation. However, Tokunaga and Bartnicki-Garcia (1971) and Grove and Bracker (1978) observed that the wall of the germ tube was continuous with the cyst wall. From our observations in direct germination of sporangia, we agree with Hegnauer and Hohl (1973). Figs. 18, 19 and 22 show that the region between the germ tube wall and the sporangial wall is the electron-dense granular structure, which is the dissolved sporangial wall. The thicker germ tube wall is evidently continuously with the thin layer wall (Fig. 22), and is not possible to be an extension of the original sporangial wall.

Two modes of sporangial direct germination, with normal germ tube on V-8 juice agar and with thin germ tube on 2% water agar, were reported (Kao and Leu, 1980). In light microscopy, the former mode is with several germ tubes in a sporangium, its germ tube is wider and able to branch, and the growth rate of the germ tube is faster. However, the later has only a germ tube which is always emerged from the papilla region, its germ tube is narrower and not able to branch, and its growth rate is slower. In electron-microscopy, no difference is observed in the cytoplasmic aspects of the both induced sporangia prior to the germ tube emergence. After that, some variances between these two modes of direct germination are conspicuous, especially for the number and the activity of the dictyosomes and the sporangial wall extension along the germ tube development. Concisely, the external and internal morphological differences between these two modes of direct germination all occur after the germ tube emergence. Significantly, the different medium results in these two kinds of germination, by normal germ tube and by thin germ tube. In other words, the external nutrient supply perhaps is the limiting factor.

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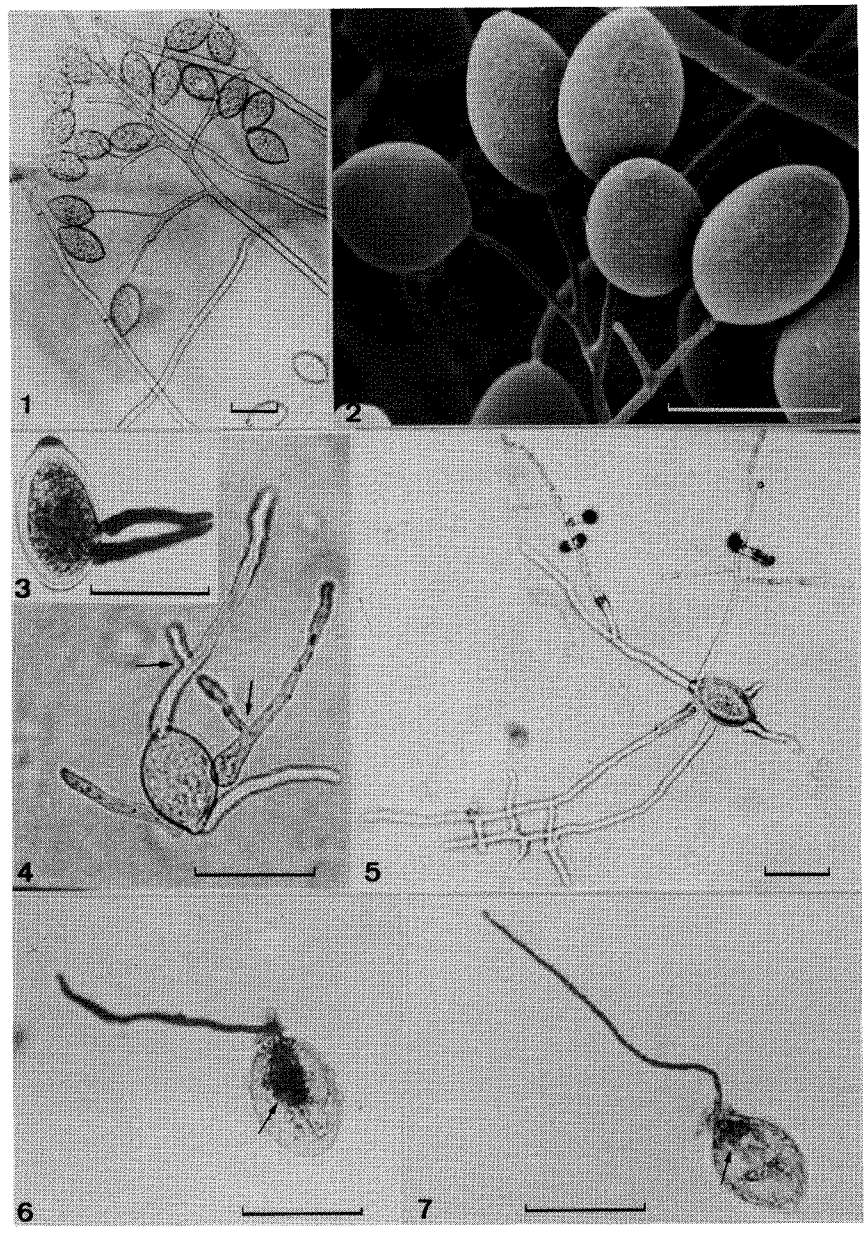
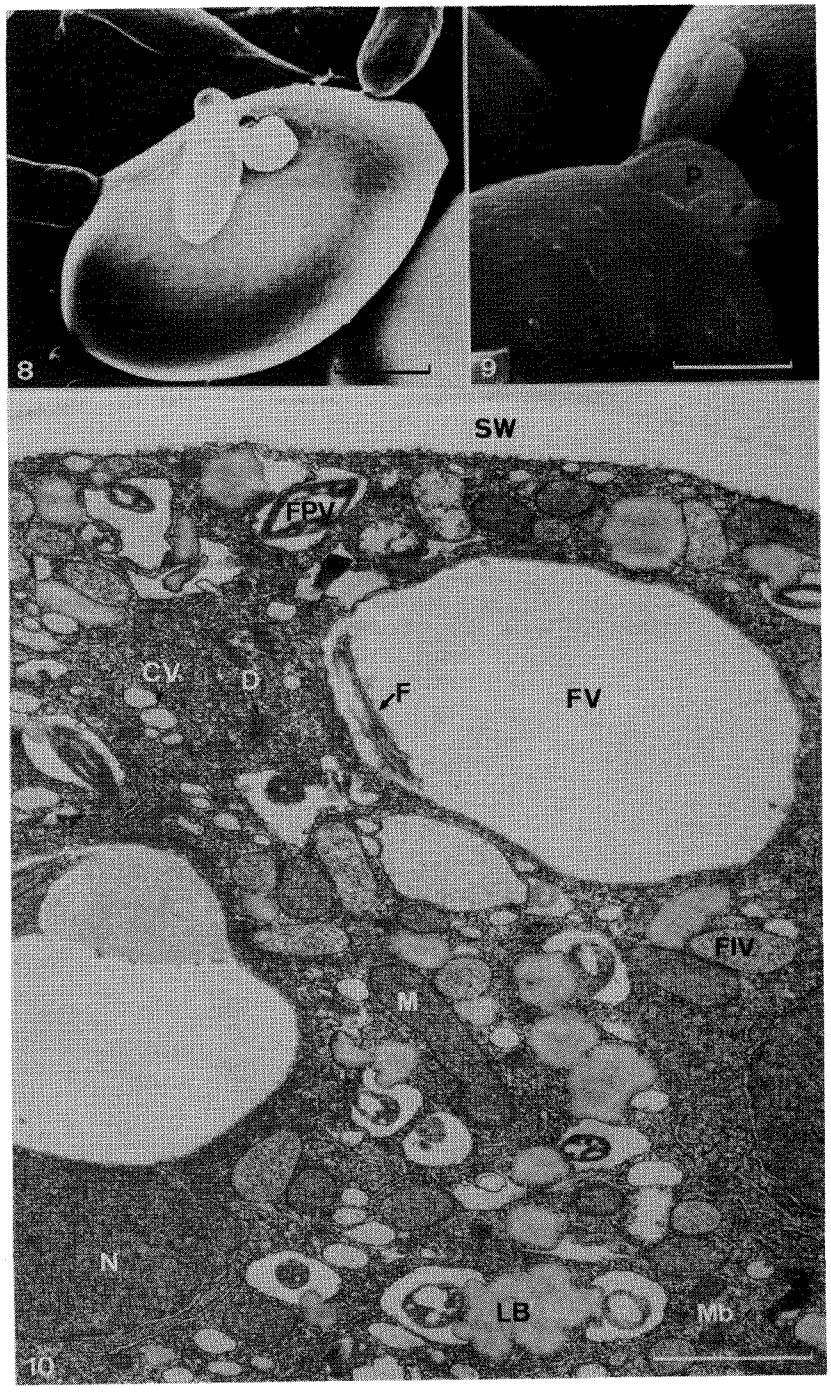


Fig. 1. Sporangiophore with detached mature sporangia. LM[4]. Bar scale=30 μm.
Fig. 2. A SEM of a sporangiophore with sporangia. Bar scale=30 μm.
Fig. 3. Early directly germinating sporangium on V-8 juice agar. LM. Bar scale=30 μm.
Figs. 4 and 5. A sporangium with several germ tubes which begin to branch (arrows in Fig. 4), later, and develop an initial mycelium on V-8 juice agar (Fig. 5). LM. All bar scale=30 μm.
Figs. 6 and 7. Direct germination of sporangium by thin germ tube at the papilla region on 2% water agar. Along the extension of germ tube, the dark materials (arrows) gradually are transported toward the germ tube. LM. All bar scale=30 μm.



Figs. 8 and 9. A view of germling with several germ tubes (Fig. 8), and the emergence of germ tube from the papilla (Fig. 9). SEM. All bar scale=5 μm.

Fig. 10. Ultrastructure of a mature sporangium containing flagella. TEM. Bar scale=1 μm.

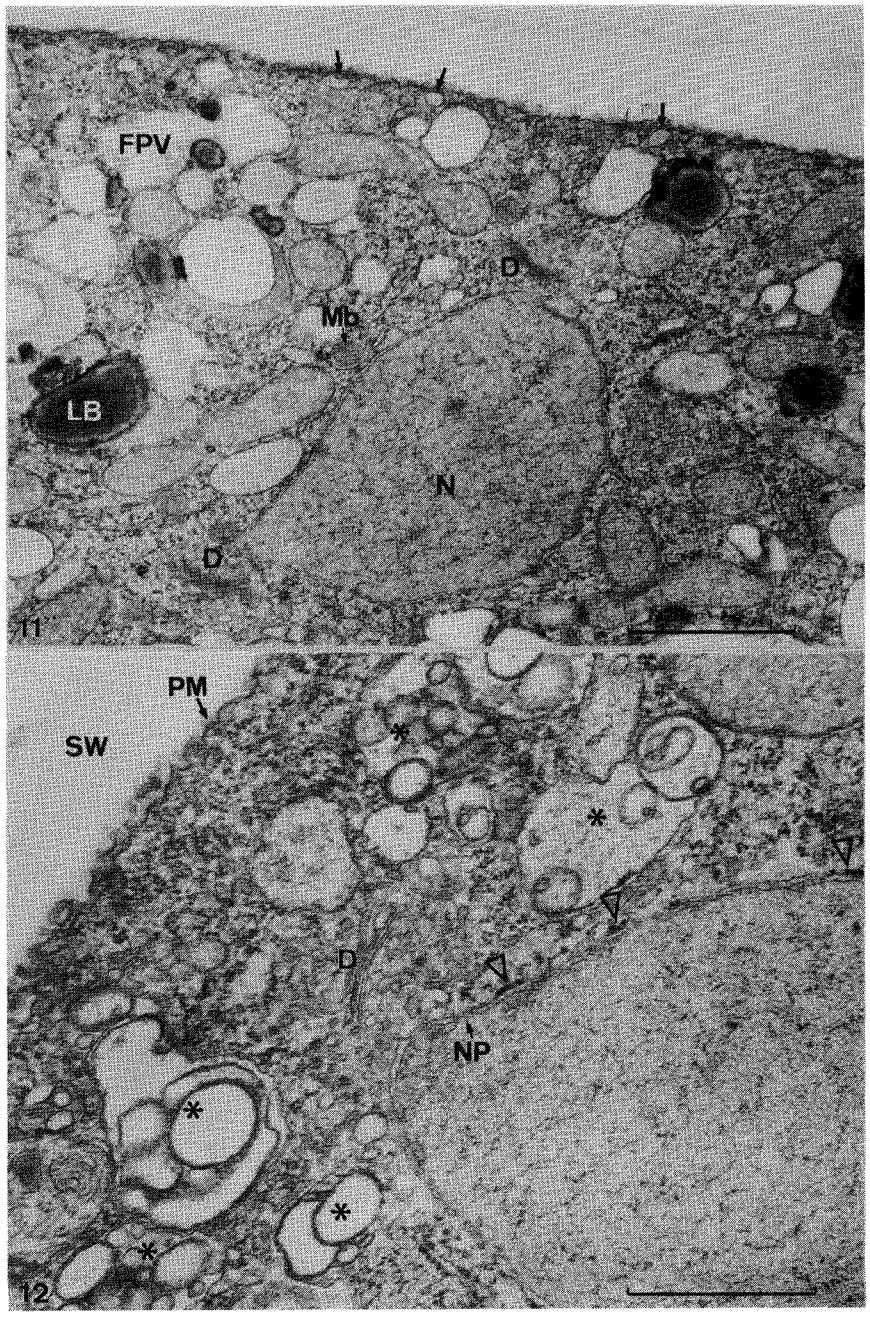


Fig. 11. Part of cytoplasm in mature sporangium exposed to V-8 juice agar before germ tube formation. Random distribution of cell wall vesicles (arrows) underlying the plasma membrane of sporangium. TEM. Bar scale=1 μm.

Fig. 12. Same as in Fig. 11. Degeneration of the flagellar vacuole and its containing flagellum (asterisks). Blank arrowheads indicate the outer membrane of nuclear envelope with dense ribosomes. TEM. Bar scale=0.5 μm.

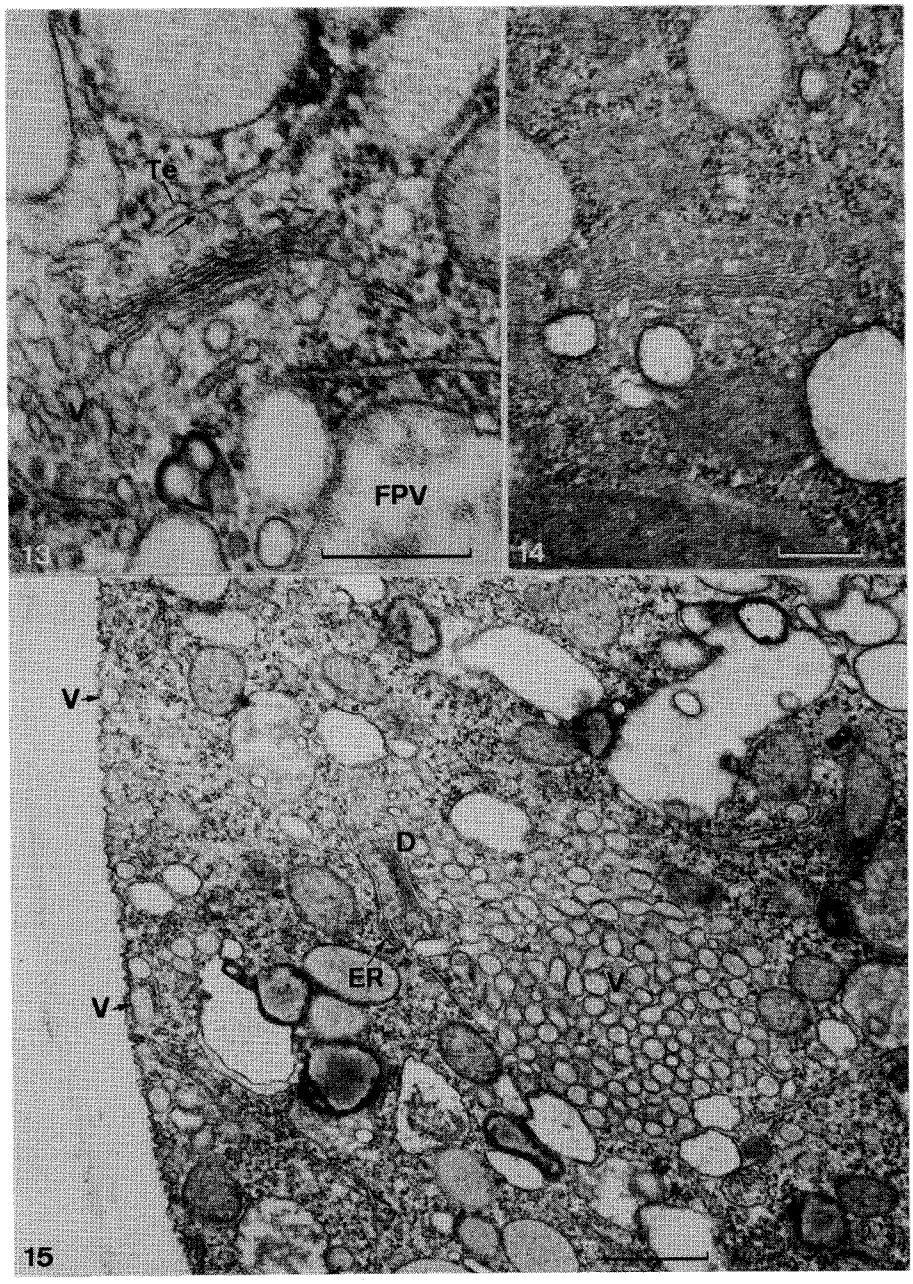


Fig. 13. Same as in Fig. 11. A dictyosome view, illustrating the presence of the cell wall vesicles associated with its ***trans*** side, vesicles which bud (arrow) from the transitional element of the rough endoplasmic reticulum on the ***cis*** side. TEM. Bar scale=0.25 μm.

Fig. 14. The reduced dictyosome in an induced sporangium (from 2% water agar) after germ tube emergence. TEM. Bar scale=0.25 μm.

Fig. 15. An accumulation of numerous cell wall vesicles near a dictyosome just prior to the germ tube formation. TEM. Bar scale=0.5 μm.

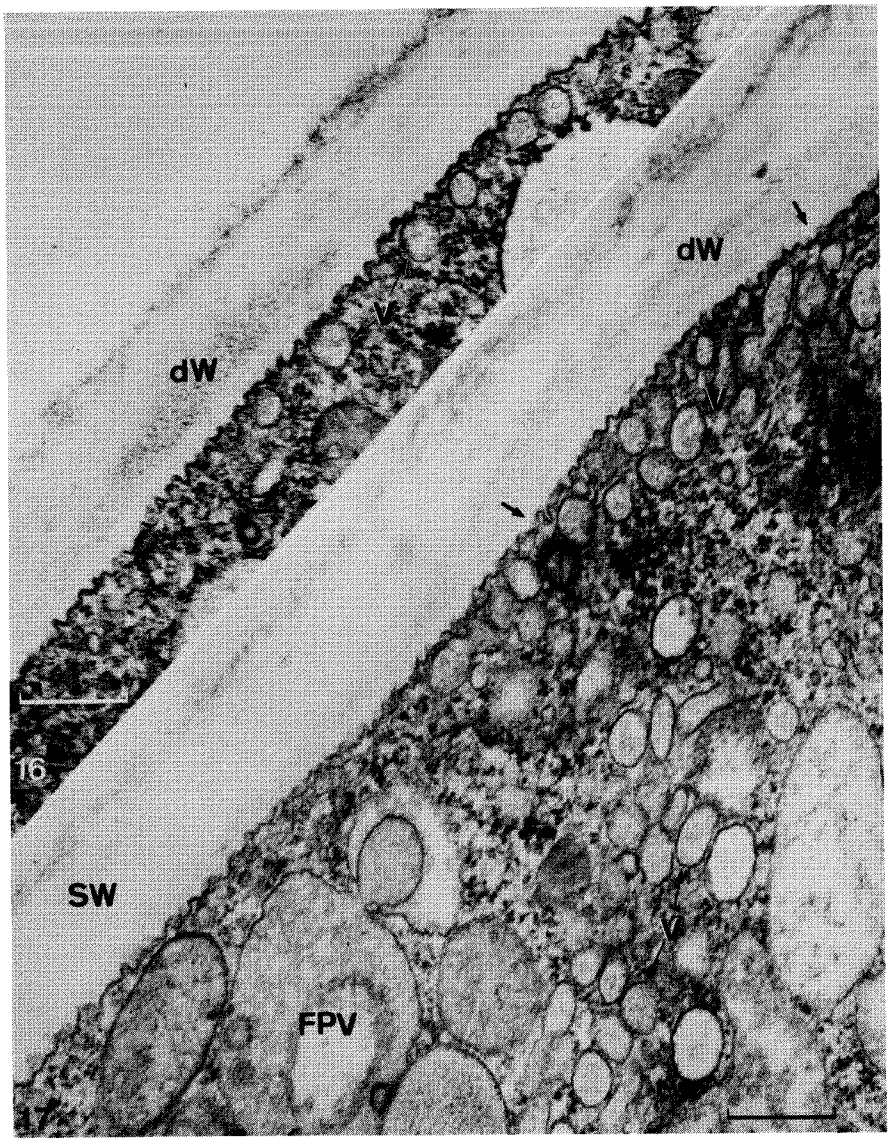


Fig. 16. Gathering of cell wall vesicles underlying the plasma membrane at the site where the original sporangial wall is dissolved into electron-dense granular structure (dW) and the germ tube is to be formed. TEM. Bar scale=0.25 μm.

Fig. 17. Part of the periphery of an induced sporangium at the earliest recognizable stage of germ tube protrusion. The incipient germ tube is recognized as a slight outward bulge in the sporangial wall beside the cluster of cell wall vesicles (comparable to the stage shown in Fig. 16). The newly deposited germ tube wall (arrows) is found between the plasma membrane and the dissolved sporangial wall. TEM. Bar scale= 0.25 μm.

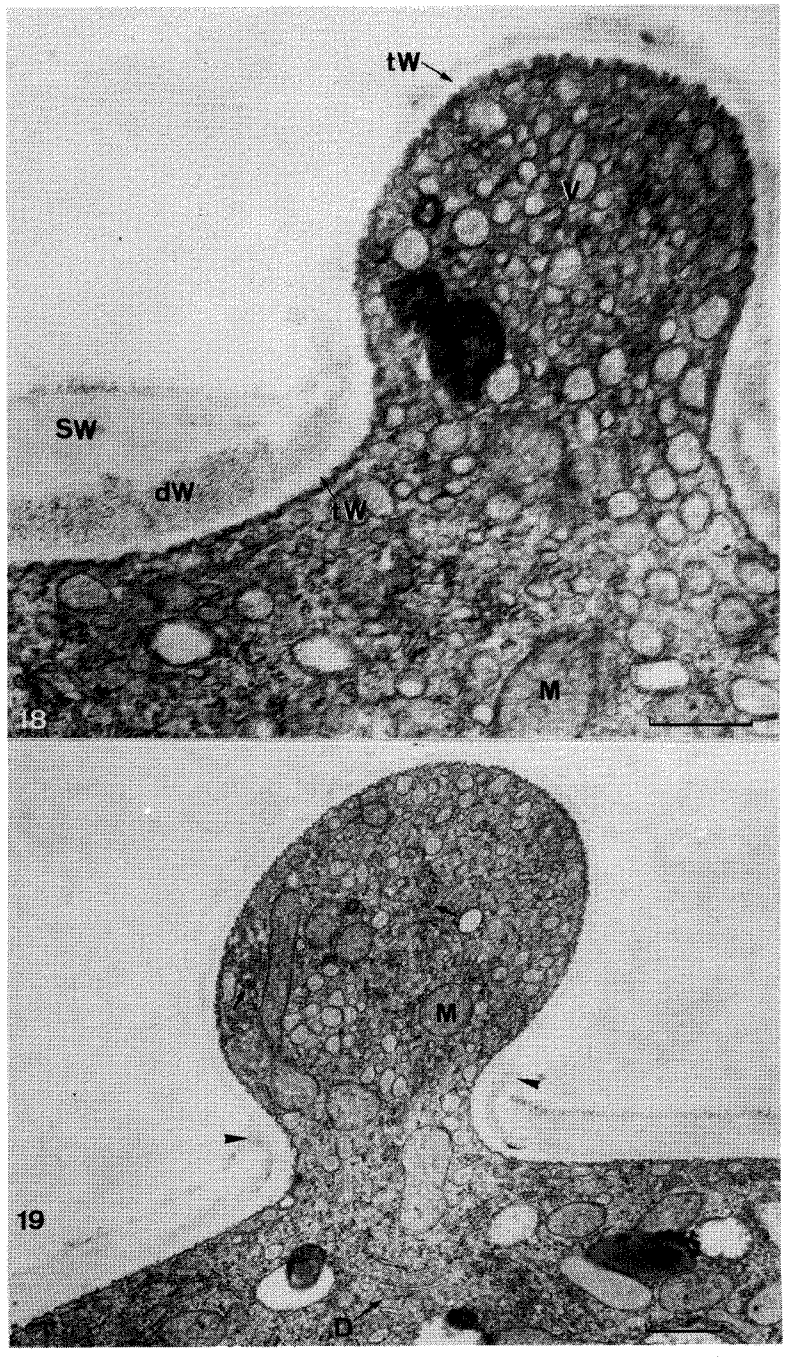


Fig. 18. The just emerging of the incipient germ tube from the sporangial wall before the swelling of the germ tube. TEM. Bar scale=0.25 μm.

Fig. 19. A more developed germ tube than that shown in Fig. 18. Segments of endoplasmic reticulum (arrows) and mitochondria have been found in the swelled cylindrical germ tube. Note the dissolved sporangial wall (arrowheads). TEM. Bar scale=0.5 μm.

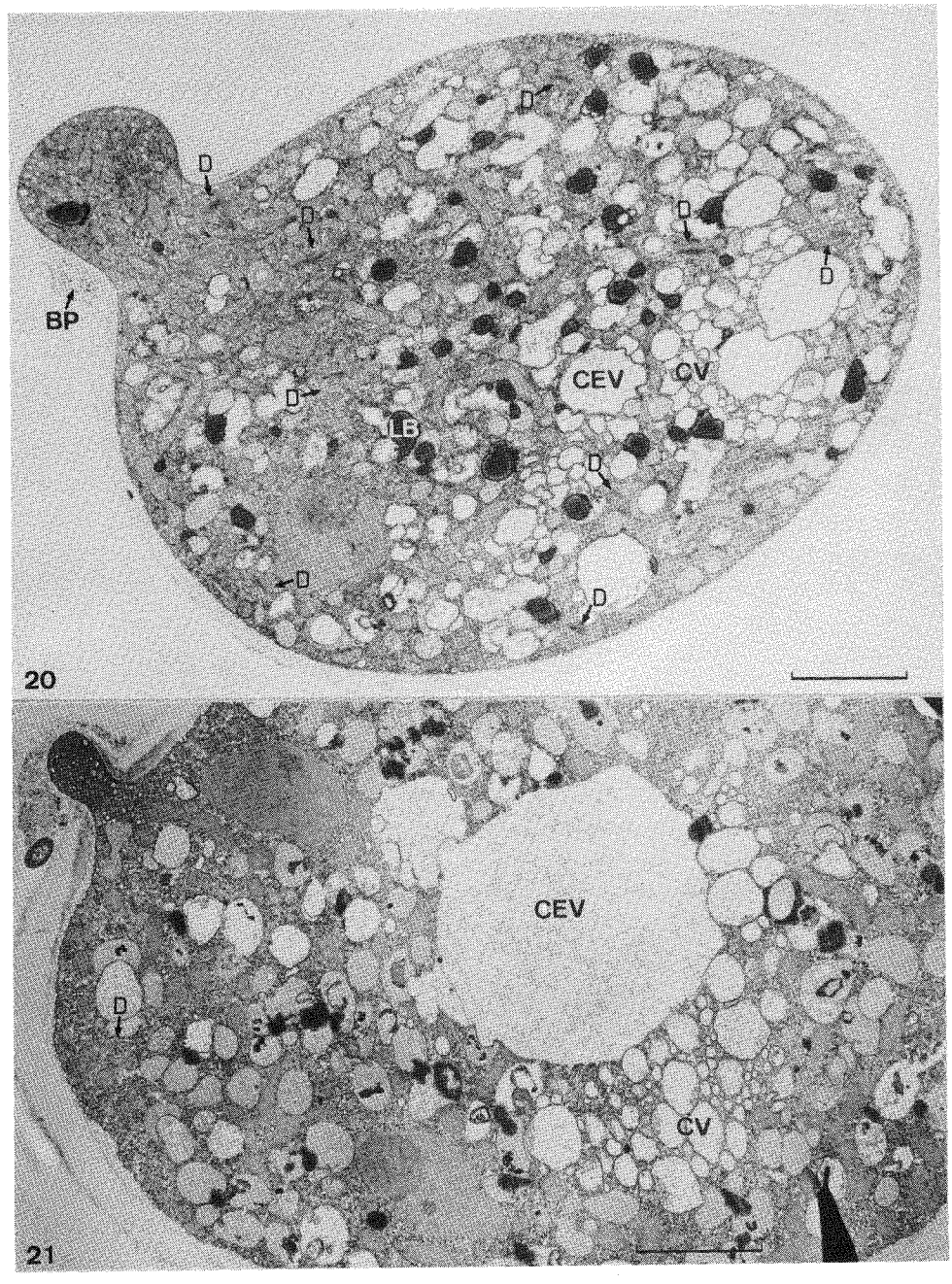
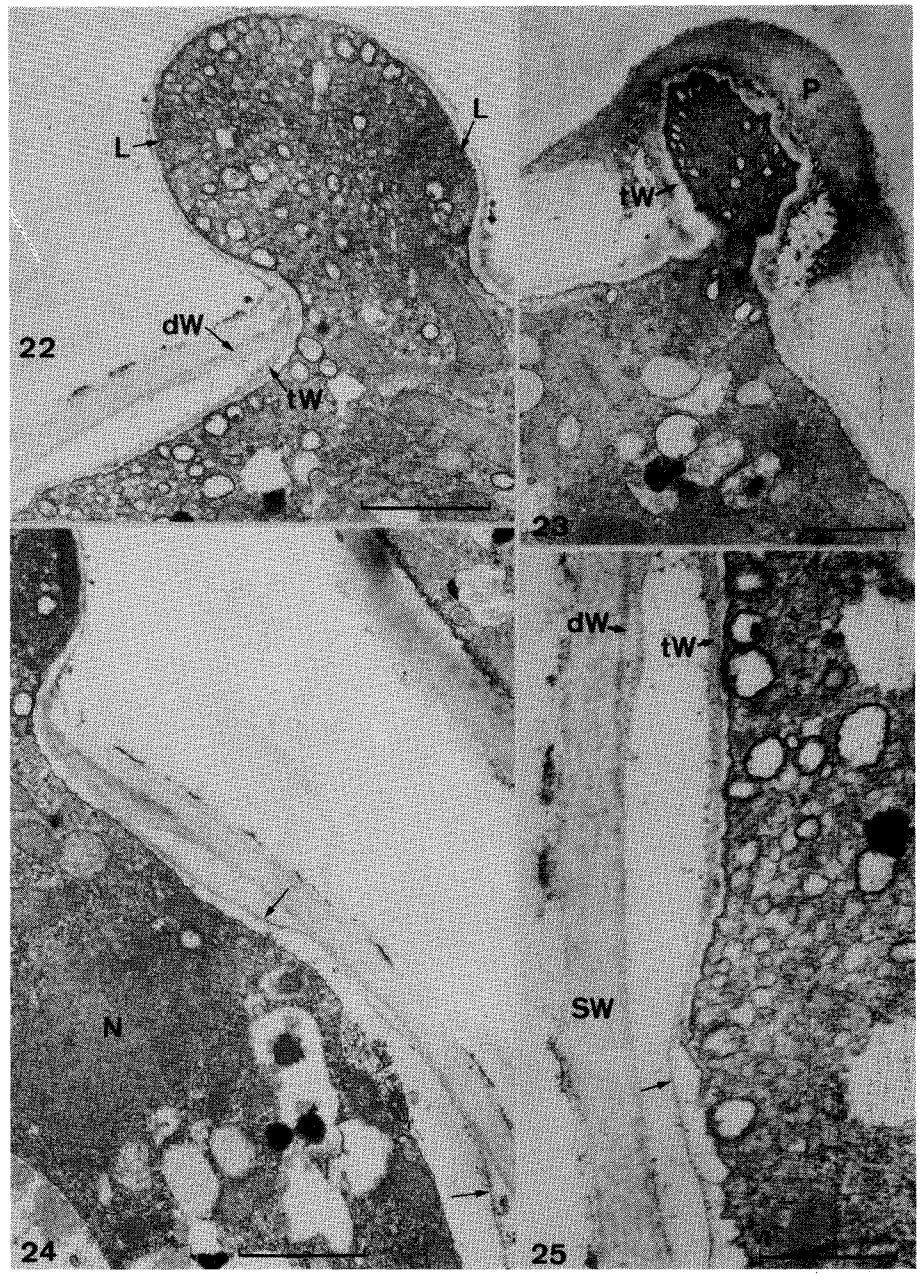


Fig. 20. A directly germinating sporangium with a germ tube (from V-8 juice agar) illustrating typical cytoplasmic organization of a germling. Nine sets of dictyosomes, elongated mitochondria and the aggregation of the cleavage vesicles and the central vacuole are observed. TEM. Bar scale=2 μm.

Fig. 21. Same as in Fig. 20, but from 2% water agar. Obviously, only one set of dictyosome is seen. TEM. Bar scale=2 μm.

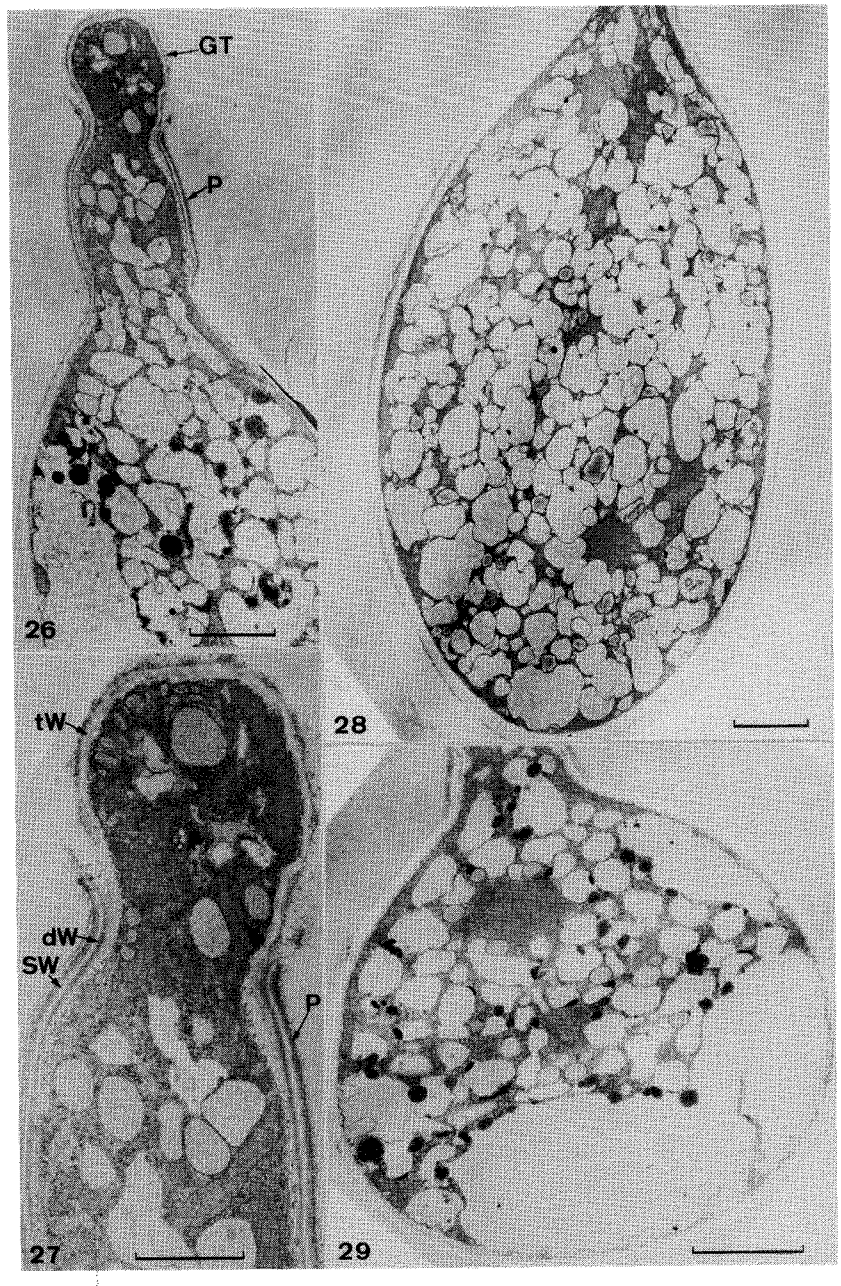


Figs. 22-29. The TEM of the directly germination sporangia on 2% water agar.

Fig. 22. A young germ tube which is filled with cell wall vesicles, including lomasomes. In addition, the sporangial wall with the dissolved sporangial wall is separated from the germ tube wall. Bar scale=1 μm.

Fig. 23. The protrusion of an incipient germ tube through the papilla. Bar scale=1 μm.

Figs. 24 and 25. The connecting of the germ tube wall with the thin layer (arrows) which surrounds the sporangial protoplast. Bar scales=1 μm, 0.5 μm, respectively.



Figs. 26 and 27. The emergence of a germ tube from the papilla, simultaneously, the papilla, and the sporangial wall extending along the development of the germ tube. Bar scale= 2 μm.

Fig. 28. Vacuolated cytoplasm in the sporangium portion of a germling after the establishment of hyphal tip. Bar scale=2 μm.

Fig. 29. A germling at the later stage than shown in Fig. 28. Large electron-transparent vacuole appears in the area back toward the germ tube. Bar scale=2 μm.

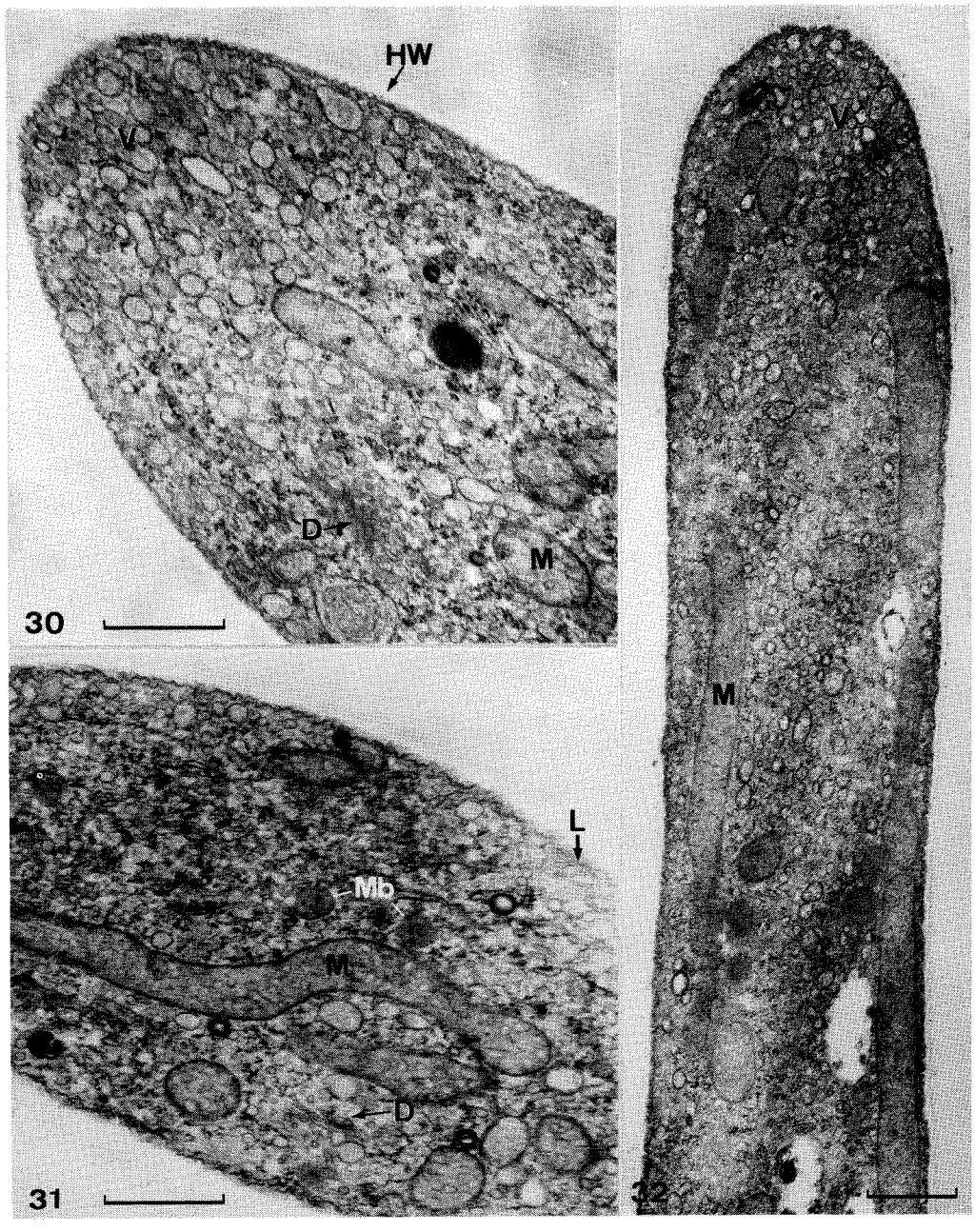


Fig. 30. Accumulation of cell wall vesicles at the hyphal tip (from V-8 juice agar). TEM. Bar scale=0.5 μm.

Fig. 31. The cytoplasm just behind the vesicular zone at the hyphal tip (from V-8 juice agar). TEM. Bar scale=0.5 μm.

Fig. 32. A near median thin section of a hyphal tip derived from a thin germ tube (from 2% water agar). TEM. Bar scale=0.5 μm.

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荔枝露疫病菌孢囊在直接發芽過程中微細構造的變化[1,2]

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荔枝露疫病菌的成熟孢囊，在 V-8 juice agar 平板上行正常的直接發芽，其內部微細構造的變化，先是鞭毛退化消失，泡囊漸集中而失去分裂作用，接着高爾基體分泌胞壁泡囊，此泡囊漸趨近且集中於孢囊壁傍之某一地區，使該區孢囊壁有部份崩解，同時初期發芽管壁在胞膜與具崩解現象的孢囊壁間形成，漸向外膨突，穿破孢囊壁，形成發芽管，此發芽管一接觸外界隨即膨大成圓柱形，進而伸長，分岐而成初期的菌絲體。發芽管伸長中，孢囊內細胞質變化，趨向於菌絲尖端的細胞質形態，即高爾基體產生胞壁泡囊供發芽管頂端之不斷生長，長形粒線體的出現，指印形泡囊的轉化成不具指印形構造的儲存性泡囊等，使得發芽管漸變成菌絲體，以達到直接發芽的目的。

本菌的成熟孢囊在2% water agar 平板上，亦行直接發芽，但與正常的直接發芽有若干差別。外部形態而言，多數孢囊具單一發芽管，且多從孢囊的乳突部位伸出，寬度較爲細瘦，且不分岐成正常菌絲體。內部微細構造而言，發芽管突出以前的孢囊內細胞質變與正常直接發芽相同，但於發芽管突破孢囊後，高爾基數目漸減且分泌的泡囊數目亦隨之降低。

[1] 本文爲臺灣植物保護中心病理組研究報告第41號

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