Scanning Electron Microscopic Study on Spore Wall Morphogenesis of *Ophioglossum thermale* Komarov. var. *nipponicum* (Miyabe et Kudo) Nishida

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Development of the spore wall ornamentation of *Ophioglossum thermale* var. *nipponicum* was studied by the scanning electron microscopy. The exospore formation may start at the beginning of the tetrad stage. The surface is smooth at first then very small pits which grow later into the crater-like ornamentation are formed. The perispore is formed at the beginning of the free spore stage. All tetrads are surrounded by sporocyte coat at earlier stage, but it has disappeared prior to the free spore stage.

Key words : Spore wall morphogenesis, *Ophioglossum*, Spore coat, Exospore, Perispore, Ornamentation.

Introduction

The ornamentation and forms of exospore and perispore of the pteridophytes is usually considered to be a specific characteristic applicable to systematics. If it is to be used as the key character of systematics, every developmental stage ought to be thoroughly analysed. However, to date, fundamental studies on the spore wall are deficient.[1-5]

This paper reports on the development of the ornamentation of exospore and perispore of *O. thermale* var. *nipponicum* observed with a scanning electron microscope.

Materials and Methods

Plants used were collected at Hachijyojima Island, Tokyo by Dr. N. Sahashi and cultivated in the experimental garden of Chiba University. The scanning electron micrographs of the spores were took with JSM-25S instrument at 15 kv and / or 20 kv.

Three kinds of samples were made by following methods ;

(1) Young fertile spikes were firstly fixed in 1 : 3 mixture of acetic acid and alcohol (AA) for about 12 hrs at −20℃, secondly in 2.5 % glutaraldehyde solution (GA) at pH 6.8 for about 24 hrs, thirdly in 2 % OsO_4 solution at room temperature for about 20 minutes. Then they were dehydrated gradually in an ethanol series and dried with the critical point dryer.

Dried spikes were sliced up into some pieces along the longitudinal axis of the spikes. These samples were mounted on the double-stick Scotch tape fixed to the brass stubs, then were coated with gold in the ion spattering instrument, JFC-1100.

(2) Some young spikes fixed with AA alone were acetolysed mildly in 1 : 9 mixture of conc. H_2SO_4 and acetic acid for about 30 minutes at 100℃, then the spores were gathered with centrifugal separator and scattered on the stubs directly. These acetolysed spores were dried in a desiccator and coated with the same method.

(3) For observation of the earlier stages of the perispore formation many tetrads and spores were scratched out on the stubs by a dissection needle from the sporangia of young spikes fixed with AA alone, and they were dried in a desiccator, then coated as described above.

Observations and Discussion

As well known, the young sporangium of *Ophioglossum* consists of seven or more cell-layered sporangium wall, two cell-layered tapetum and central sporogenous cell mass. Then the tapetum and the central cell mass change into the plasmodium and the spore mother cells, respectively. And these cells become the tetrahedral spores after meiotic divisions.[6, 7]

In the materials fixed in AA, GA, and OsO_4 solutions, the polyhedral archesporial cells of very young sporangia are covered with thin cell wall on which there are many small granules. The size of these granules ranges from 0.01 μm to 1.00 μm (Fig. 1).

The surface of many spore mother cells at the free cell stage is covered with many small granules less than 0.1 μm and net-worked thin fibrils. The thickness of these fibrils are less

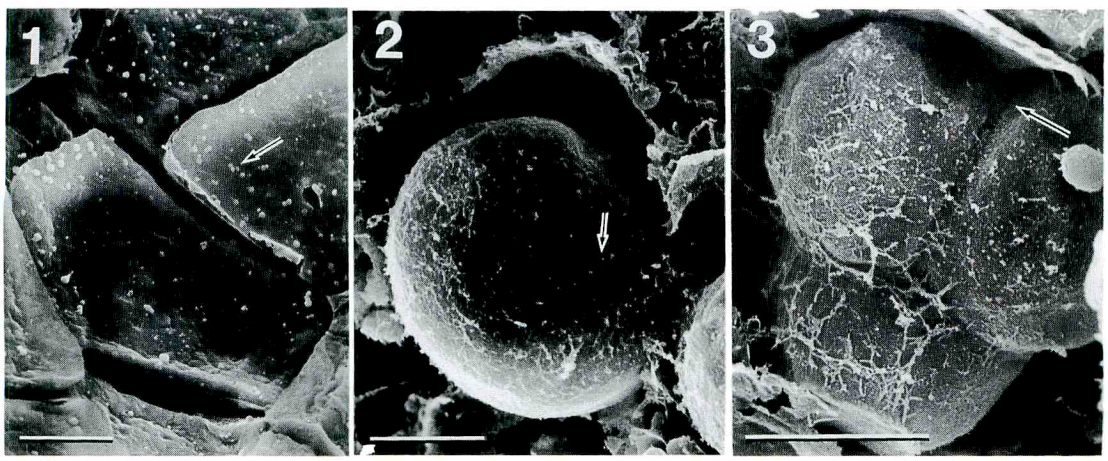


Fig. 1. Archesporial cells with small granules. Arrow points the granules and white bar indicates 10 μm.
Fig. 2. Spore mother cell surface with granules and fibrils. Arrow points the granules and bar indicates 10 μm.
Fig. 3. The beginning of the tetrad stage. Arrow indicates the belt-like structure. Bar indicates 10 μm.

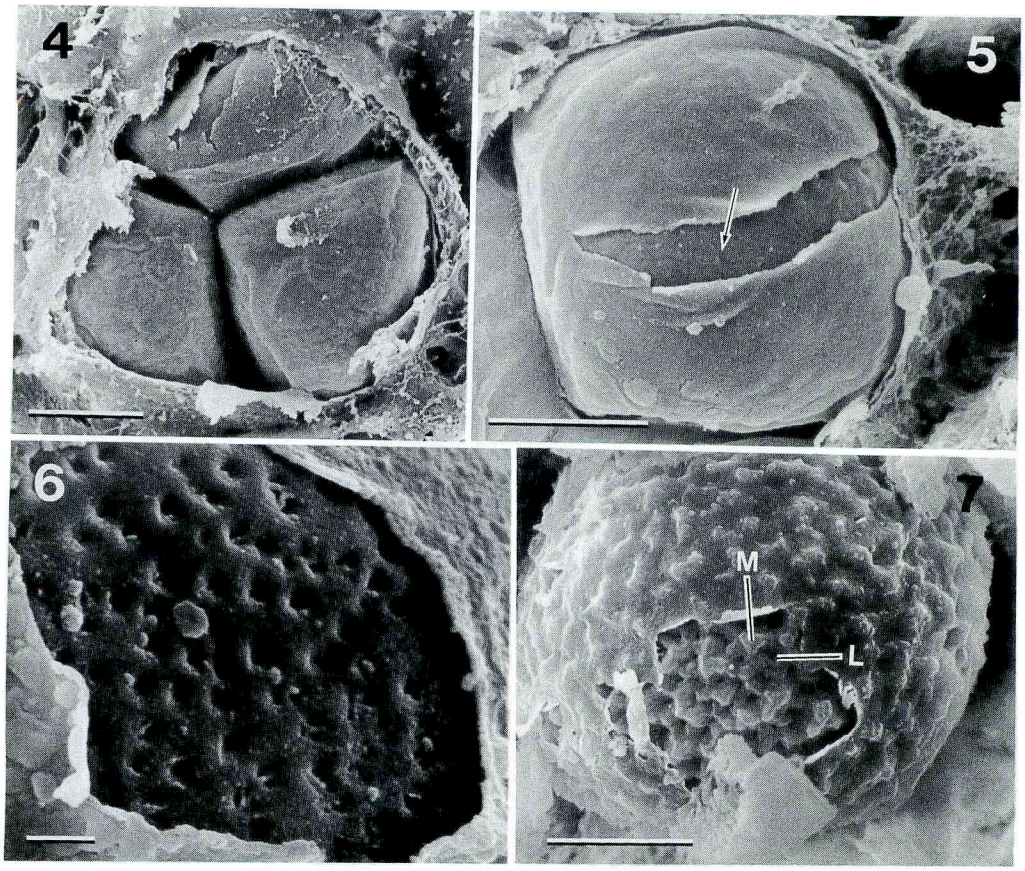
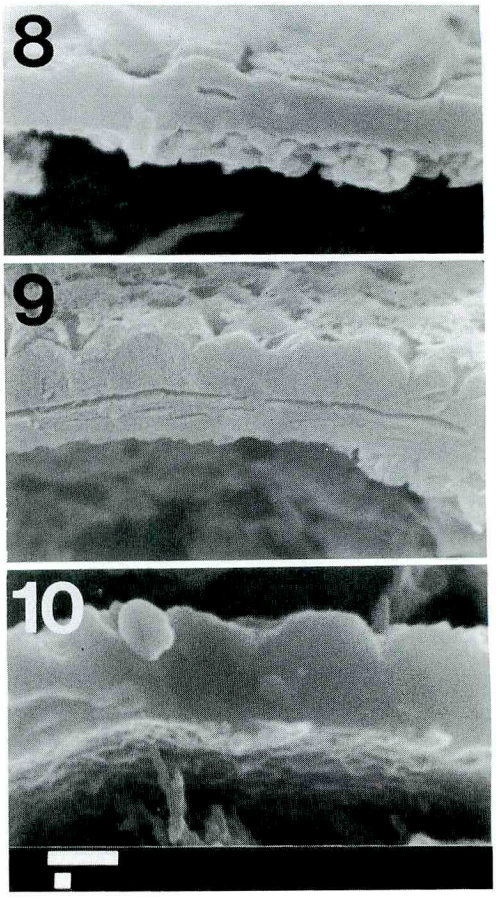


Fig. 4. A tetrad covered with sporocyte coat and plasmodium. Bar indicates 10 μm.
Fig. 5. One of four cells of a tetrad, showing the sporocyte coat and exospore surface on which the very small pits are observed. Arrow points the pits. Bar indicates 10 μm.
Fig. 6. The sporocyte coat and exospore surface with many crater-like structure at the end of the tetrad stage. Bar indicates 1 μm.
Fig. 7. A free spore, showing an ornamentation of muri (M) and lumina (L). The thin membrane may be the perispore. Bar indicates 10 μm.

than 0.1 μm (Fig. 2). This outermost layer of the spore mother cells may be the primary sporocyte coat which was observed in transmission electron micrographs.[5] I could not observe any dyad period at the present investigation, but found many cells at tetrad stage. At the beginning of tetrad stage, four cells of a tetrad are connected with the belt-like structure (Fig. 3, the arrow indicates this structure). The surface structure of these cells are very similar to those of the spore mother cells. The sectioned figures obtained by TEM at this stage show the very complicated thin-lamellated structure of the exospore.[1, 3, 5] Later, near the end of this stage, the sporocyte coat or callose membrane surrounding the exopore is clearly observed (Figs. 4 and 5). There are many small pits on the surface of the young exospore. The diameter of these pits is less than 0.01μm, and the thickness of exospore at this satge is about 1μm as shown in Fig. 8. At the end of this stage, the pits on the exospore surface have developed into the crater-like

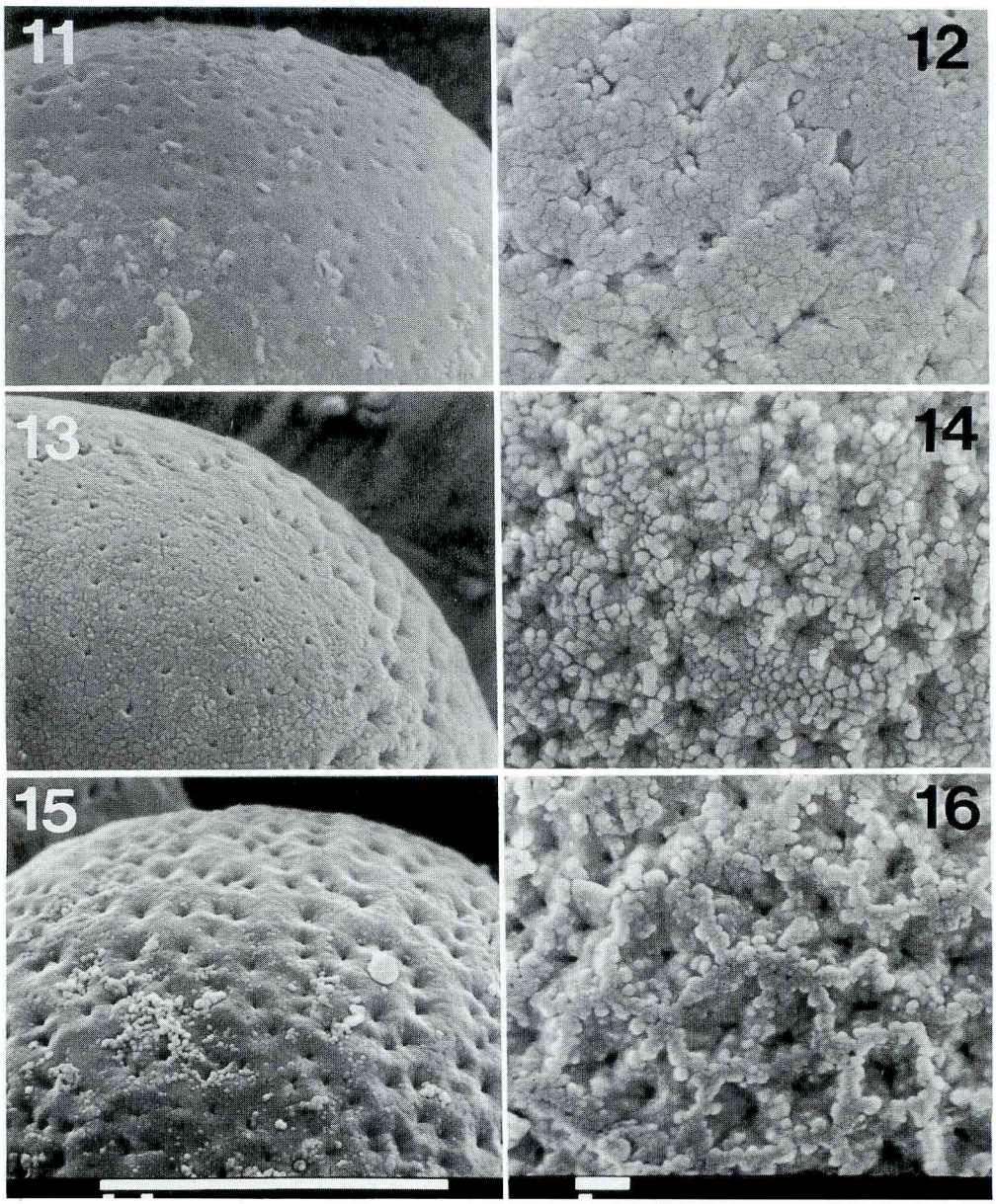
structure of about 0.5 μm in diameter (Fig. 6). The frequency of the pits is 50 ($\pm$5) per 100 μm^2 of exospore surface. The exospore thickness at this stage is about 1.5 μm having many small clefts or thin and flat sack-like structure (Fig. 9). Such a structure has already been reported using TEM by Lugardon.[1, 3] At the beginning of the free spore stage, the fundamental ornamentation of exospore has been formed, namely the typical muri and lumina structures have well developed. And somewhat later very thin perispore which is less than 0.1 μm in thickness has been formed (Fig. 7). At this stage, the thickness of the exospore becomes more than 2 μm in the distal region (Fig. 10). According to the observations under the TEM, this perispore is not a simple remnant of the sporocyte coat but it is formed immediately after the solution of the spore coat.[5] There are many fine granules ranging from 0.05 μm to 0.01 μm in diameter on the surface of the perispore (Fig. 7). On the matured and dried spores, we cannot find this thin perispore under the SEM, because they adhere to the exospore surface closely as will be mentioned later.



Figs. 8–10. Cross sectioned exospore, showing its successive developmental stages (cf. text). White bar indicates 1 μm.

In the samples obtained by the second method including the acetolysis treatment, the sporogenous tissues before the tetrad stage such as spore mother cells and dyads may have been melted down because of nonexistence of the sporopollenin in their wall structure. So we could not observe them by this method. On the other hand, the method is suitable for observing clearly the surface structure of the developing exospores because the tapetal substance, sporocyte coat, and perispore can be excluded by this treatment.

The early developmental stages of the exospore are shown in Figs. 11 to 15. These figures clearly demonstrate successive formation of the exospore ornamentation of this species. The figures 12 and 14 which are highly magnified exospore surface correspond to a certain part of the figures 11 and 13, respectively. In very highly magnified microphotos obtained by SEM (Fig. 12), the exospore surface at early developmental stage seems to be composed of granulous substances ranging from 0.1 μm to 0.5 μm in diameter. This structure, however, may be an ar-



Figs. 11-16. The early developmental stages of exospore observed in the materials acetolysed. White bars in Figs. 11, 13 and 15 indicate 10 μm, while those in Figs. 12, 14 and 16 indicate 1 μm. (cf. text).

tifact originated in the air-dry method judging from the figures obtained by critical point drying method and TEM.[1, 2, 5] The figure 16 is a certain part of the exospore surface of a matured spore prepared by acetolysis method. Typical muri with ridge and lumina or crater-like structure are observed. This ornamentation is almost universal in the family Ophioglossaceae, though there are some specific modifications.[8, 9]

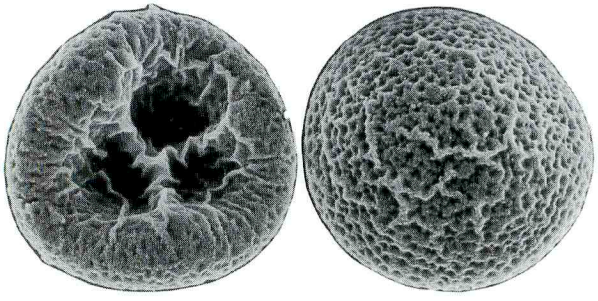


Fig. 17 and 18. Young spores covered with the viscous perispore, which were scratched out from young sporangia by dissection needle. Proximal (17) and distal view (18). Bar indicates 10 μm.

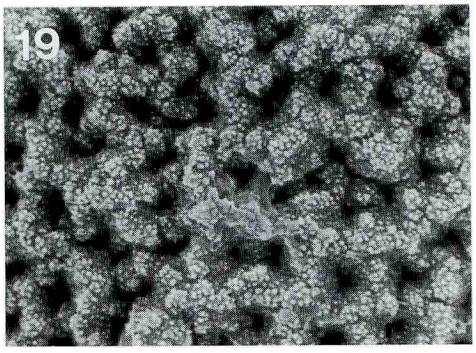


Fig. 19. A part of distal surface of a mature spore. Perispore adheres tightly on the exospore surface. Bar indicates 1 μm.

An earlier stage of the perispore formation can be observed on premature spores which scratched out by dissection needle from the sporangia of the young spikes fixed with AA alone. It seems to be very thin viscous membrane (Figs. 17 and 18). However, at the maturity, this membrane may adhere tightly on the granulous and reticulated exospore surface (Fig. 19).

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