Studies on the Germination of the Spores in Some Mosses.

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(*Received Nov. 11, 1958*)

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The reproduction of mosses is chiefly made by means of spores. To begin with, every spores puts forth the protonema system, and the formaiion of gametophytes is brought forth by the germination of spores and regeneration of gametophytes. In order to clarity the mechanism of reproduction in *Barbula unguiculata, Bartramia crispata, Mnium microphyllum, Pogonatum inflexum, Dicranum japonicum*, the author have undertaken experiment on the germination of spores.

Methods

The cultures of the spore were made on the plates and filter paper and slides as well. The treated filter papers were placed on the absorbent cotton in closed Petri-dishes which poured Benecke's nutrient solution, keep in the upper surfaced over the solution. The nutrient solution bearing different pH value was prepared and was renewed with every two weeks during the experiment. At the same time they were also made on 1% agar Benecke's nutrient media mounted on slides as mentioned above. The culture furnitures and media were sterilized in Koch's stream-sterilizer, preceding the experiment. All these cultures were kept at room temparature and placed near a window illuminated by day-light, avoiding any direct rays of the sun.

Results

Barbula unguiculata Hedw.

In Matsue, the spprogonium of this species ripened during late April and early May. The materials collected from the stone wall of basalt, on 17th of May 1957, were wrapped up in the paraffine paper and kept in the desicator. The spores were sown on the agar nutrient media, bearing a pH value of 8, on 21st of September of the same year. The spores of this species were large, measuring about 12 μ in diameter. The spores on the media swelled to a considerable size, measuring 20μ in diameter within 2 or 3 days after the treatment. The chloroplasts in the endospores much increased in three or four days after the treatment. Five or six days after the treatment, the exospores ruptured

and a germination tube appeared. The germ tubes were cut off from the endospores by a cross wall and then developed into chlorophylliferous filament. In many cases, the germ tube occured from the one side of the spore but very rarely an another germ tube developed from the opposite side of spores the same time. (Fig. 14) These filaments showed positive phototrophic character. (Fig. 14) In respect to the width of the filaments, and the acount of chloroplasts which are contained in the filamentous cell, there were not found any differences between both the main filaments. When the main fila-

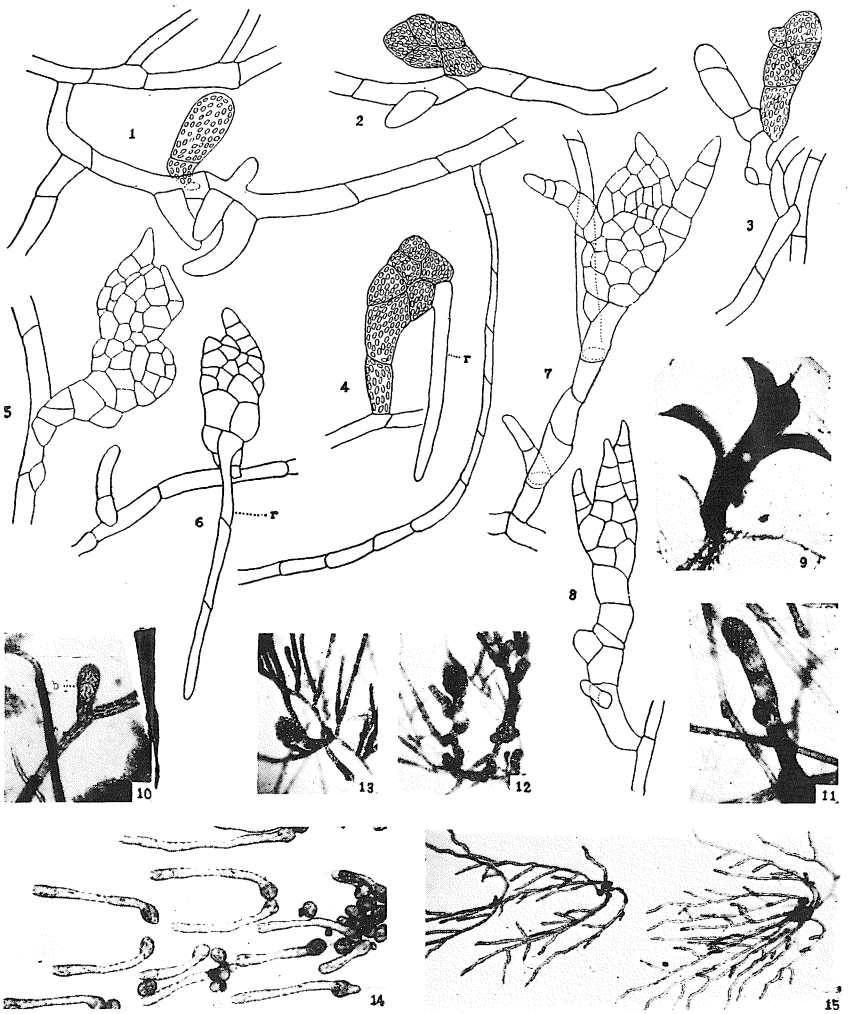

Fig. 1 Germination of spores in Barbula unguiculata Hedw. (1–15)
1–8, early stages in development of bud, ×180
9, leafy plant, ×50
10–12, bud of leafy plant (10. 11...×110, 12...×60)
13, globose-mass like gametophore, ×60
14, germination of spores, ×125
25, filaments showed positive phototrophic character, ×60
r...rhizoid, b...bus. (Yuko Nishida)

ment acquired 6 or 7 cells' length the branches were formed from the upper part of the main filament. These branch filaments showed also positive phototrophic character. (Fig. 15). In many cases, the branches were produced from the point just anterior to the cross wall of a filamentous cell. There were two kinds of branch filaments; the one crawled on the nutrient media and another grew apart from the media. Several huge protuberances were found dear the forked points of these filaments. These protuberances developed new filaments, bearing oblique cross walls, and their width are about 2.5 times as large as ordinary branch filaments which grew from the main filaments. These filaments turned into the rhizoid-like filaments. The protonema attained about 2cm. in length at the end of October, and no were elongation of filaments occurred. Thus, the protonema system was formed. The bud of leafy plant appeared on the main filaments and on the branch filaments attached the media on about 45th day after treatment. No buds were found on the branch filaments which elongated towards the air. The buds appeared as a huge protuberance on the point anterior to the cross walls (Fig. 10). These protuberances are similar to the Indian-Club in shape and are formed from a terminal cell and a stalk which holds the cell. The stalk consisted of 3 or 4 cells arranged in a chain. This was different from that of *Bartramia crispata* which is formed from a single cell. The terminal cell developed on the stalk cell contains abundant chloroplasts and later forms a globose cell mass by successive segmentation. Several long rhizoids grew from the basal part of a young shoot. Thus, the leafy plant grew to the size that can be recognized by the naked eyes at the middle of November (Fig. 9).

Bartramia crispata Schimp.

The spores were collected on 19th of April, 1957, and sown on the media on the 14th of May of the same year. Within 4 days after the treatment, one rarely two germ tubes appeared from the spore through ruptured exospores. The germ tubes were soon cut by the cross wall and formed a branch filament by successive cell divisions. There may be recognized two types in the branch filament; the one had colorless cell walls and the cross walls occured at right angle to the longitudinal axis and contained numerous chloroplasts, the other had brown cell walls and oblique cross walls and contained few chloroplasts. The branch filaments developed in various directions and occasionally some of them turned into brown rhizoid-like filaments, bearing oblique cross walls. Thus, a protonema system was formed in about 30 days after the treatment. In the middle of June, a cell of the main or branch filaments produced protuberance on the point just anterior to the cross wall (Fig. 18). The cell derived from the protuberance developed into a

leafy plant. The buds of leafy shoot are ovoid and their cells contain rich cytoplasm and numerous chloroplasts. At first a diagonal septum was set in the cavity of the initial cell of a leafy plant and was followed by two or three additional diagonal ones and thus divided an apical cell by three divided faces. The rhizoids were produced from the basal part of a young leafy plant, but it occurred after the formation of two or three leaves. The rhizoids had a numerous spots on the surface of brown cell walls, oblique cross walls and few chloroplasts.

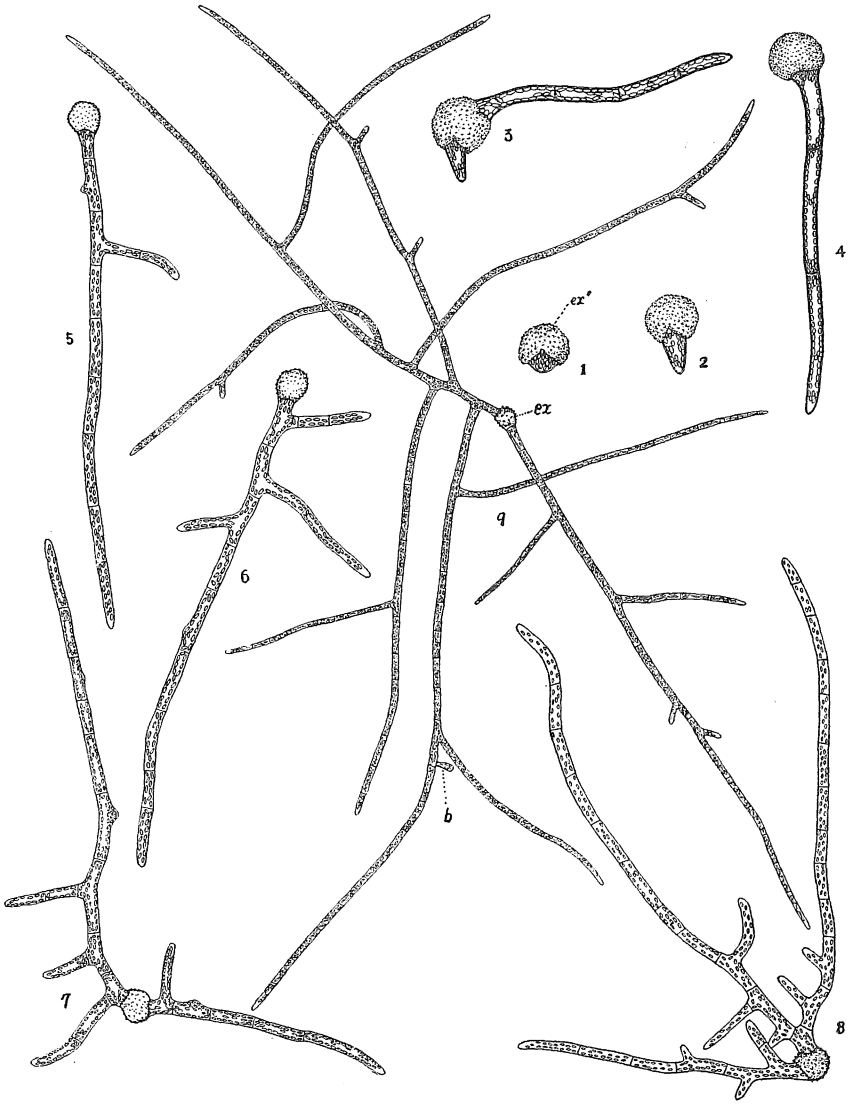

Fig. 2 Germination of spores in *Bartramia crispata Schimp.* (1-9)
1-8, Germination of spores (1-4, ×200 5-8, ×150)
9, protonema system, showing bud of leafy plant, ×25
ex ... exospore, b ... bud of leafy plant. (Yuko Nishida)

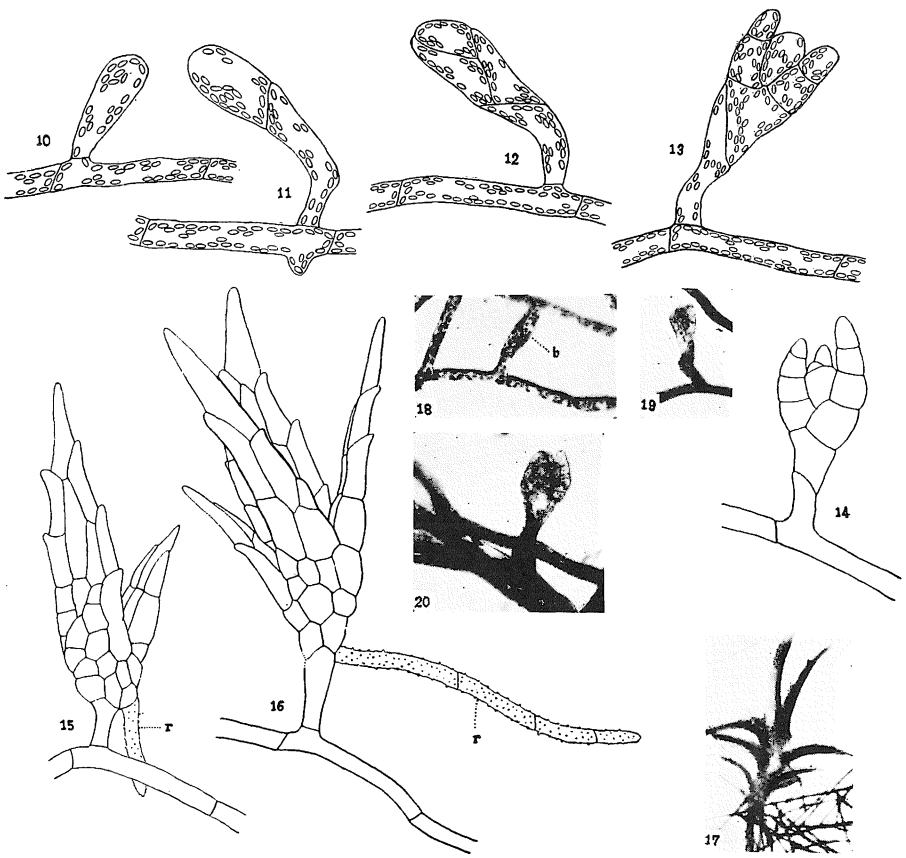

Fig. 3 Germination of spores in *Bartramia crispatr Schimp.* (10–20)
10–14, early stages in development of bud, ×200
15–16, a leafy plant with a rhizoid, ×250
17, ditto, ×40
18–20, showing a bud of leafy plant on the protonema, (18, 20×150, 19×110)
r ... rhizoid, b ... bud. (Yuko Nishida)

Mnium microphyllum Doz. et Molk.

The spores were collected on April 10, 1957 at Sotonakahara-machi, Matsue city and kept in dry condition until the culture experiments were undertaken. They were sown on the porous plates and filter papers absorbed Benecke's nutrient solution, bearing a pH value of 6 on 14th of May of the same year. The spores of this species were large, measuring about 22μ in diameter. They were swollen whithin a few days after the treatment and enlarged by 35μ. The endospores produced one or two germ tubes. The germ tube was soon divided by a cross wall and developed into a multicellular filament (Fig. 3). When the filament attained 10–15 cells' length several branches were formed

on the apical part of the main filament. In June, the main and branch filaments increased rapidly their length and showed positive phototrophic character. Two kinks of branches were recognized; the one ran on the surface of the substratum or raised the substratum, and the other crept into the substratum. The former bore colorless, cross walls at right angle to the longitudinal axis and contained numerous chloroplasts, the latter was brown and beared oblique wall, and contained chloroplasts. The branches crept into the agar turned into rhizoidal filaments. The growth of the rhizoids on the porous plates was better than the filter papers. Thus, a protonema system was formed

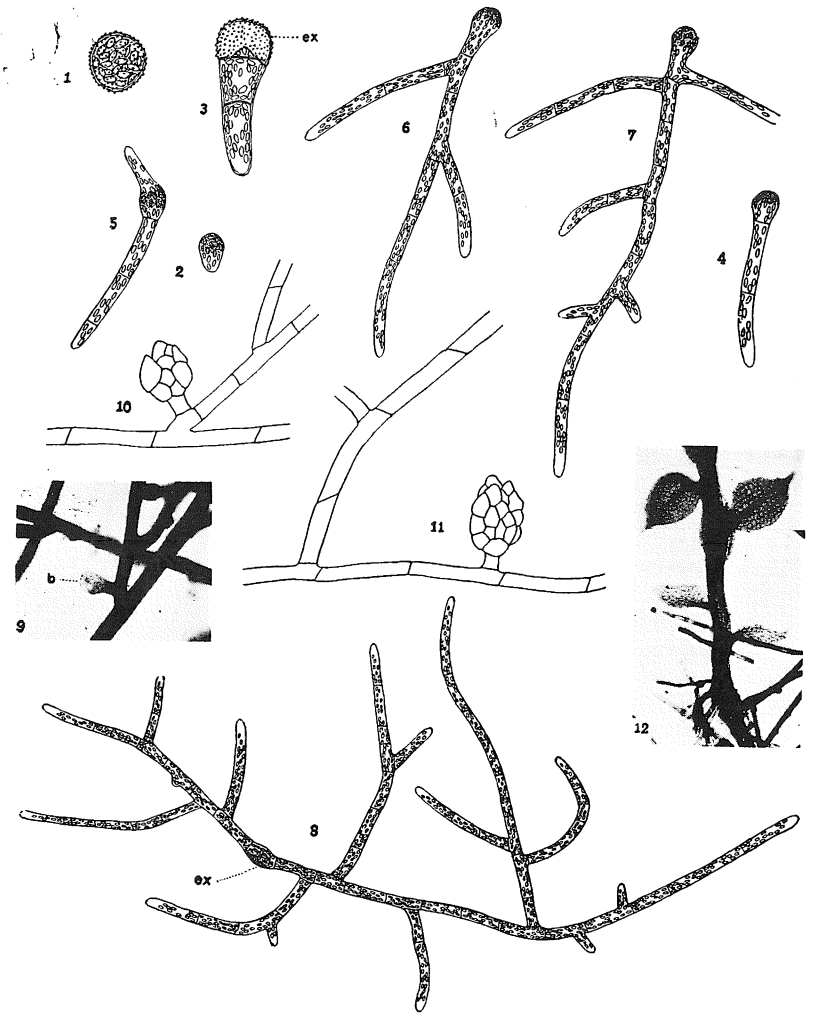

Fig. 4 Germination of spores in *Mnium microphyllum D. M.* (1–12)
1–7, germination of spores, (1. 3. ×300 2. 4. 5. 6. 7. ×150)
8, showing a protonema system, ×50
9, showing a bud of leafy plant on the filament, ×150
10–11, early stages in development of a bud, ×200
12, showing a leafy plant developed on filament of protonema, ×40
ex ... exospore, b ... bud. (Yuko Nishida)

whithin two months after the treatment. Several huge protuberances were found on the main and branch filaments. As is seen in Fig. 9, this protuberance developed as the bud of a leafy plant. Through the successive segmentation of this cell a leafy plant was established.

Pogonatum inflexum Ldb.

The materiales were got on 16th of October, 1957 in Mt. Daisen, Tottori Pref. The spores were wrapped in the paraffine paper and kept in the desicator. They were cultured on the porous plates submerged in the Benecke's nutrient solution bearing a pH value of 6, on 5th of November of the same year. The spores of this species were about 6–8μ in diameter. The spores on the porous plates were swollen and became about 10μ in diameter within two weeks after the treatment. The spores germed in the middle of November. The feature of the germination was similar to that of the preceding species. In late November, the germ tube developed into 3–4 cells' length but no further development of the filament was observed during the winter. In early March of the following year, they regained activity and became longer and branched as time went on. The filamentous cells were very slender, measuring about 10μ in width and contained rather few chloroplasts. The branch filaments were extended on the surface of the substratum for a short distance and turned into the air, showing the positive phototrophic character (Fig. 6). The branch filaments reached to about 2cm in length. Thus, a protonema system was formed, but no buds of leafy plant were found both on main and branch filaments during one year of the culture.

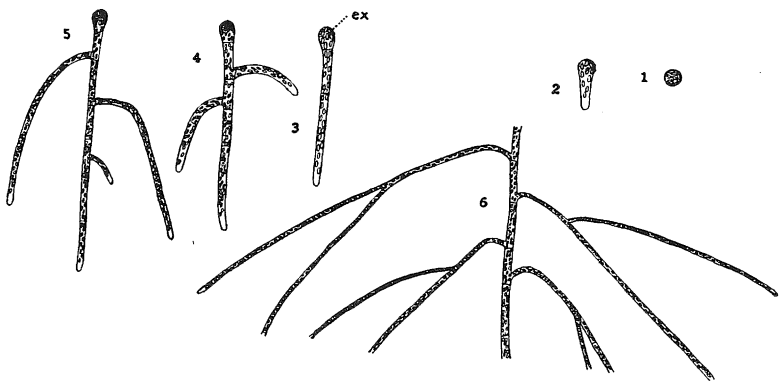

Fig. 5 Germination of spores in *Pogonatum inflexum Ldb.* (1–6)
1–5, germination of spore, ×250
6, showing branch filaments, ×200
ex ... exospore. (Yuko Nishida)

Discranium japonicum Mitt.

The spores were collected in September 20, 1957, at Rakuzan-park, Matsue city. The spores were scattered on the porous plates submerged in the Benecke's nutrient solution, bearing a pH value of 6, on 11th of October of the same year. The endospore emerged as a protuberance through the rupture of exospore after two weeks but the growth of the filament was stopped during the winter. In early April of the following year, the filaments regained activity and increased their length and produced several short and chlorophyllose branch filaments from the upper part of the main filament. There are two kinds of branch filaments similar to those of the preceding species (*Mnium microphyllum*, *Barbular unguiculata*). The branch filaments elongated toward the air, showing the positive phototrophic character, and attained about 1.5cm in length. In late October the bud of leafy plant appeared at the point just anterior to the transverse walls of the filamentous cells (Fig. 5, 6). The cells of bud contain rich cytoplasm and numerous chloroplasts. At the end of October, 1958, a single juvenile plant bearing well formed leaves and rhizoids formed on a protonema (Fig. 8).

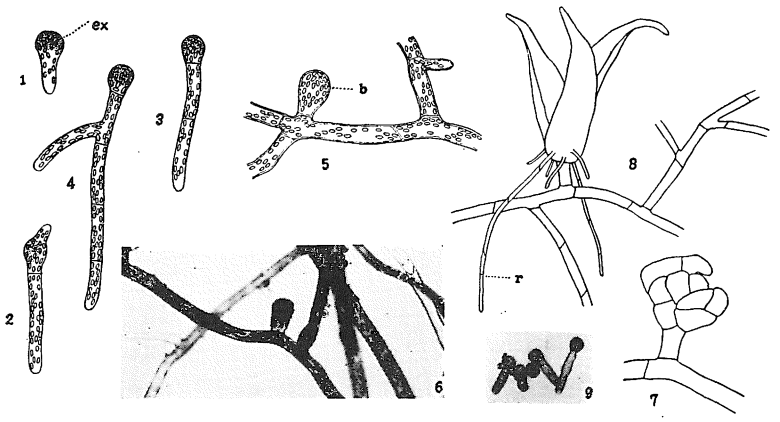

Fig. 6 Germination of spores is *Dicranum japonicum Mitt.* (1-9)

1-4, germination of spore, ×100

5, a bud of leafy plant developed on protonema, ×125

6, ditto, ×100

7, early stage in development of a bud, ×125

8, a leafy plant bearing rhizoids and a filament on protonema, ×70

9, germination of spores, ×60

ex ... exospore, r ... rhizoid, b ... bud. (Yuko Nishida)

Summary

The spores of *Barbula unguiculata, Bartramia crispata, Mnium microphyllum, Dicranum japonicum* need one or two weeks for germination. They elongated germ tube from one or two sides of spore and developed into the filamentous protonema. These filaments showed the positive phototrophic character. In culture experiment in Spring the protonema system was formed in about two months respectively. The bud of leafy plant of *Barbula unguiculata, Bartramia crispata, Mnium microphyllum, Dicranum japonicum* grew on the main and branch filament which crawled on the substratum but in *Pogonatum inflexum*, no buds of leafy plant were found during one year of the culture. The buds produced as a protuberance, in which contained rich cytoplasm and chloroplasts.

Acknowledgement

This research, subsidized by the Science Department of Ministry of Education, was directed in full by Mr. Yuko Nishita of the Lower Secondary School attached to Faculty of Education, Shimane University. The authors (Yuko Nishida and Shintaro Saito) wish to express many thanks to Dr. Akira Noguchi, professor at Kumamoto University, for his suggestions and warmhearted helps afforded to this work.

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