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A STUDY ON THE CHARACTERISTICS OF SPORE GERMINATION AND PROTONEMAL DEVELOPMENT IN *LINDBERGIA BRACHYPTERA*

ИЗУЧЕНИЕ ПРОРАСТАНИЯ СПОР И РАЗВИТИЯ ПРОТОНЕМЫ *LINDBERGIA BRACHYPTERA*

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Abstract

The spore germination, protonemal development and gametophyte differentiation of *Lindbergia brachyptera* are observed in cultivation. The results show that the germinating time of the spore is 3 days, and the germination pattern is exosporous. When the culture is 8 days old, the germinating rate of spores is 95%. The protonemal system of *Lindbergia brachyptera* consists of two elements, the filamentous chloronema and the rhizoid. The initial of gametophytes are observed to have differentiated from the chloronema.

Резюме

Изучено прорастание спор, развитие протонемы и ранние стадии формирования гаметофора у *Lindbergia brachyptera*, выращиваемой в культуре.Прорастение споры экзоспоровое, происходит за три дня. За восемь дней в 'посеве' не агаре прорастает 95% спор. Протонемная система *Lindbergia brachyptera* состоит из двух элементов: нитевидной протонемы и ризоидов. Гаметофоры развиваются на нитях хлоронемы.

INTRODUCTION

The development from a spore to protonema is the first step of growth in mosses. The research on spore germination, protonemal development and their sporeling growth helps significantly with the study on the phylogeny, relationships among various groups, and the evolution of the generative system in mosses (Hu 1987, Nishida 1978). Besides, the information provides the theoretical basis to understand fully the biology of bryophytes and to study the spore germination and protonemal development in large scale culturing application. Therefore, it is of great significance to carry out research on spore germination and protonemal development of bryophytes.

For many years, Japanese researchers have made intensive studies in this field. In 1970s, Nishida (1978) had illustrated the spore germination and protonemal development of sporelings of 121 species of mosses, 58 of which (from 26 families and 47 genera) were reported for the first time. In comparison, there is less research done in this area in China. Gao & Zhang (1986) studied spore germination and protonemal development of 9 species of Bryidae found in China, 7 of which were firstly reported. Bao & Cao (2000,2001) gave a detailed description of the transition from spore germination to mature gametophyte of *Sphagnum* and *Marchantia polymorpha* by way of microphotographs. The experiments conducted by Zhang & al. (1995) indicated that spore germination and protonemal development are influenced by pH. Zhao & al. (2002) and Fan & al. (2003) published on the spore germination and protonemal development of more than 30 species of bryophytes, such as *Physcomitrium eurystomum, Entodon macropodus, Pylaisia polyantha, Encalypta ciliata*, and 13 of them were also the first reports.

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MATERIAL AND METHODS

Experimenting materials

Mature spores of *Lindbergia brachyptera* (Mitt.) Kindb. were collected from Mt. Xiaowutai, Hebei Province (voucher specimen: *Huang Shi-liang 030004*, 1 Sept 2003, HBNU). This species is monoicous and grows on the topsoil of rock, particularly on trunks in the birch and pine forest. In China, *L. brachyptera* is commonly distributed in Hebei, south of Mt. Xing-an, Hu-xi Plateau, Mt. Helan, Yunnan, and Tibet, and also in Japan, Europe and North America. Among its congeners, two distinctive characters of this species are the verrucose leaf cells and the percurrent leaf costa.

The experimental agar substrate contains modified Knop's solution (Table 1). The agar powder used in this study was produced by SIGMA.

Procedures

(1) Preparation of substrate: The Knop's solution was prepared according to Table 1, and mixed with the agar until the concentration reached 2%. The mixture was then melted together. Afterwards, the solution was poured onto the 60 mm sterilized culturing container to solidify.

(2) Preparation of spore fluid: On the Aseptic Bench in the laboratory, mature capsule of *L. brachyptera* was dipped in 75% ethanol and subsequently cleansed 5 times by distilled water. The cleaned capsule was opened by a sterile nipper and a dissecting needle,and the spores were mixed with 10 ml distilled water to make the spore fluid.

(3) Inoculation of spore fluid: The spore fluid was poured onto the solidified agar substrate with the use of a pipettor.

(4) Incubation of spores: The experimental set ups were placed in the RXZ controlled growth chamber for incubation. The temperature was 20±2°; relative humidity was more than 80%, illumination intensity, 24 $lux \cdot m^{-2} \cdot s^{-1}$, and the illumination time, $12h \cdot d^{-1}$.

(5) Inspection of spore germination: The growing condition of the spores was observed everyday. Microphotography was done when needed. Illustrations of representative germination of the moss spore were undertaken, noting the germination process particularly. The observation of the spore germination was made under the 10×10 microscopic eyepieces. Throughout the period, germinating spores were randomly selected for observation, and the calculation of the percentage of germination was done by the average of 3 times observations.

Table 1. Modified recipe of Knop's solution (pH=7.0)

Reagent	Concentration ($mg \cdot L^{-1}$)
$Ca(NO_3)_2 \cdot 4H_2O$	1000
KNO_3	250
KH_2PO_4	250
$MgSO_4 \cdot 7H_2O$	250
$ZnSO_4 \cdot 7H_2O$	3
$FeSO_4 \cdot 7H_2O$	12.5
$NaNO_3$	Subtle
Distilled water	1L

RESULTS

The germination pattern of the spores of *L. brachyptera* was observed to be exosporous. Spore is spherical to subspherical, opague, and flavo-green in colour. Papillae were observed on the surface of the spore whose diameter is about 25 m (Figs. I: 1; II: 1). Oil droplet and some undeveloped chloroplasts could be seen inside the cell.

The observation made under the compound microscope showed that the process from spore germination to gametophyte formation in *L. brachyptera* is composed of three distinct stages, namely, the spore germination, protonemal development, and differentiation of the gametophyte.

1. Spore Germination

Spore germination was observed to be divided into two consequential stages: (1) the spore swelled in the presence of water, the exine broke and the intine became disrupted subsequently. Along with the process, the inner structure of the spore changed; the most distinctive character of which was the amount of chloroplast increased distinctively. The average diameter of the spore by now measured about 28 μm; and (2) the first sign of spore germination was the appearance of protruding protoplast and, at the same time, the putting forth of a filamentous germ tube (Figs. 1: 2; 2: 2&3).

The results showed further that after 4 days, the germination rate was 53.9%; after 5 days, the germination rate was 58.9%. The polarity of spore germination was observed to be single and double (Figs. 1: 3; 2: 4); spores with three germinating poles were rarely observed.

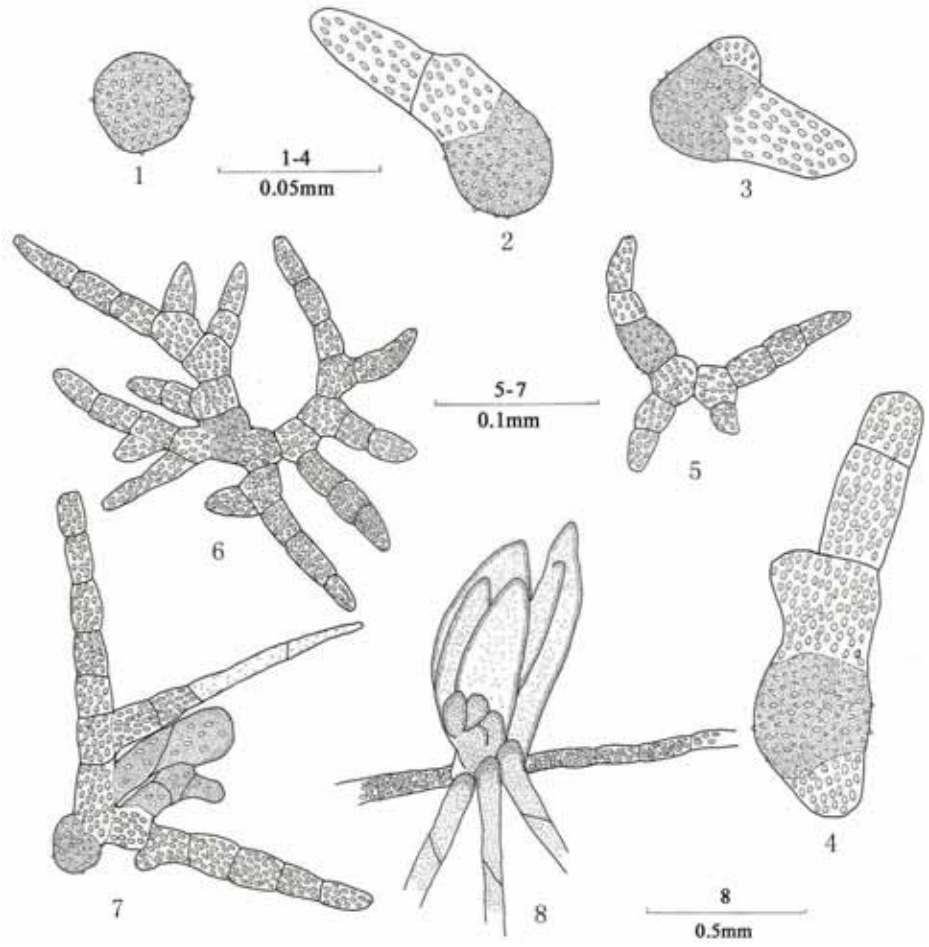

Fig. 1. 1: Spore; 2: Spore showing the first division; 3: Spore with bi-polar germination; 4-5: Filamentous chloronema differentiating into primary branches; 6: Second branches growing from primary branches; 7: The apical cell of filamentous chloronema differentiating into rhizoid and the basal cell differentiating into gametophytic initial cell; 8: Young gametophyte.

2. Protonemal Development

When chloronema grew to 65~70 m long, it differentiated into a primary or main branch (Figs. 1: 4&5; 2: 5-7). On the 7th day, the germination rate of spores reached 84% and the spores with two germinating poles were about 16 %. However, by then, there were still immature brown spores which did not germinate. When filamentous chloronema grew to four cells, 15 filamentous individuals were randomly selected to measure their length, showing a mean value of 105 m. On the 10th day, spores with three germinating poles started to emerge, and the maximum length of filamentous chloronema became 180 m, which was composed of 7 cells. Later on, the chloronema growth accelerated. On the 12th day, more primary branches were differentiated from chloronema and more secondary branches grew out from them (Figs. 1: 6; 2: 8-9). On the 15th day, the chloronema became highly and diffusedly branched, and the apical growth slowed down (Fig. 2: 10). On the 23th day, the apical cells of chloronema began to differentiate into rhizoid, whose cross walls were oblique and transparent. Rhizoid then grew rapidly and its cells were slender.

3. Gametophyte Differentiation

The protonemal system of *L. brachyptera* began to produce gametophyte at the end of the above two stages. Between the 24th day and 25th day, an initial cell of the gametophyte appeared at the base of filamentous chloro-

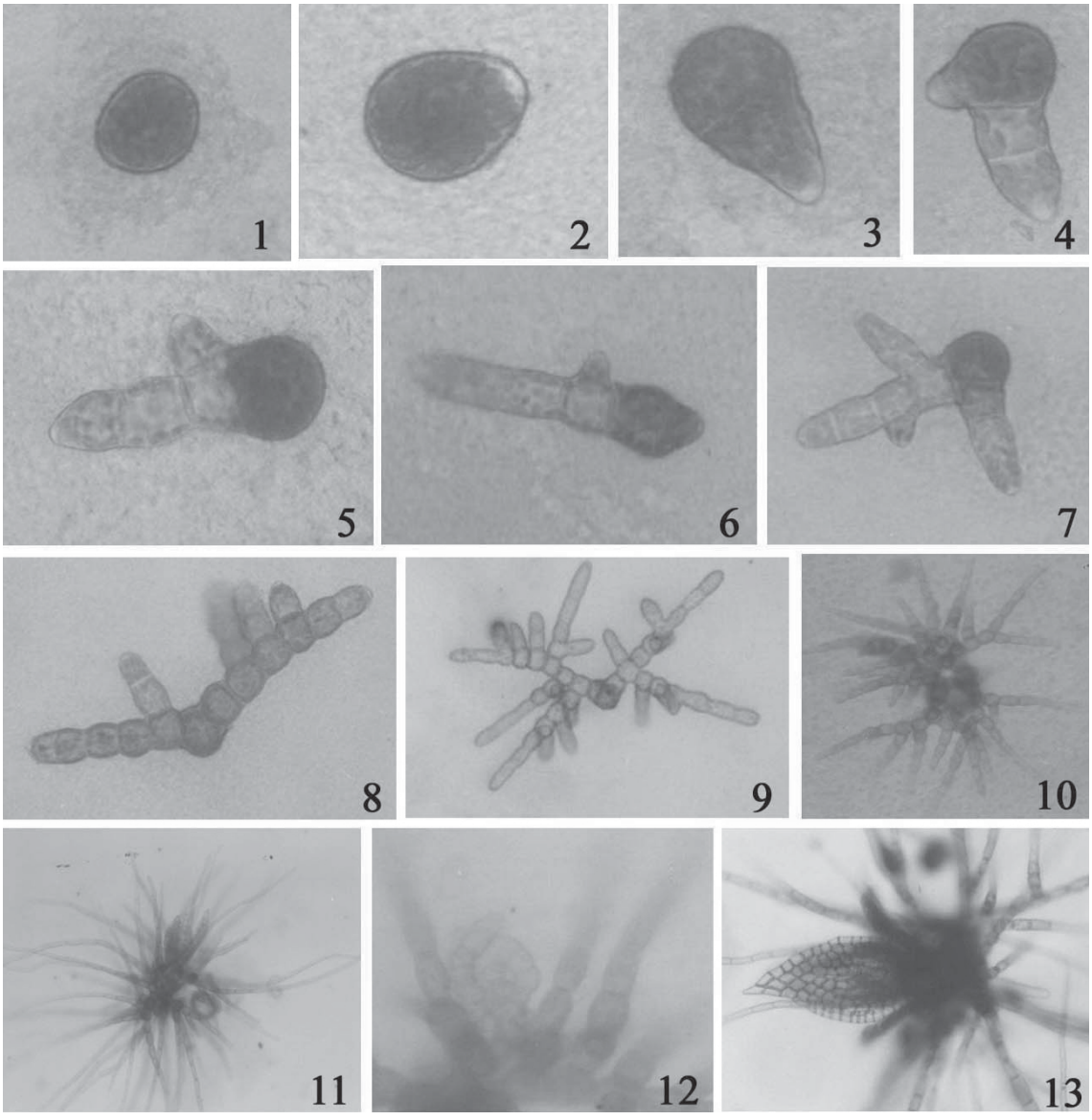

Fig. 1. 1: Spore; 2-3: Spore showing the first division; 4: Spore with bi-polar germination; 5-6: Filamentous chloronema differentiating into primary branch; 7-9: The base of the branchy filamentous chloronema; 10: The apical cell of filamentous chloronema differentiating into rhizoid; 11-12: The basal cell of filamentous chloronema differentiating into gametophyte initial cell; 13: Young gametophyte. Magnifications: 7,10~11,13, ×9; 6,8-9,12, ×18; 1-5: ×36.

nema (Figs. 1: 7; 2: 11-12). It was observed as a single round swollen cell which was bigger than the filamentous chloronema at the beginning. This cell enlarged and divided to form a multi-cellular mulberry-like structure and the amount of chloroplast increased two days later. The cell then divided in vertical, horizontal and tangential directions, forming a group of meristematic cells. At the same time, many aerial-chloronema grew away from the substrate. On the 28th day, the gametophyte had already formed 2-3 leaves and the slightly transparent rhizoid arose at the base of the gametophyte. The rhizoid had oblique walls and did not contain any chloroplast. The gametophyte continued to produce 3-5 leaves in the following days (Figs. 1: 8; 2: 13). On the 35th day, the number of leaves reached 5-7 and new gametophyte cells appeared at the bottom of the former

gametophytic initial. At this time, the basal cells of chloronema became stumpy and moniliform, and they showed a deep-green appearance. Because the gametophyte does not grow at the tip of the protonema, the continued development of the protonemal system is not affected.

ANALYSIS AND DISCUSSION

The substrate used to culture *L. brachyptera* in this study is agar with a pH value of 5.8. Tile and filter paper have been used as substrates in other studies, but not in the present experiment. Under the former experimental condition using the filter paper, observation by the microscope is not easy. Furthermore, the protonema system is easily damaged in handling, which is harmful to the successive observation. Moreover, the chemicals in the filter paper tend to influence the development of protonema.

The temperature in this experiment is kept at 20±2°. This temperature range has been shown to be a suitable condition for the spore germination, protonemal development and gametophyte differentiation. If the temperature is a bit higher, it is easy for the bacteria to grow and reproduce on the culture medium; in contrast, a lower temperature harms the development of the protonema.

Spore germination

The spores of *L. brachyptera* still germinated 6 to 10 months after collected from the wild. The results of our study showed that temperature, humidity, culture medium, pH, and illumination, have direct influences on the germination rate, protonemal development and the morphological character of the protonemal system.

The germination of the spore of *L. brachyptera* is of the exosporous type. The germination polarity is mostly 2–3 poles, and less, with a single pole germination; although occasionally, some spores were observed to have four poles of germination. The protonemal system contains two parts, one is the filamentous chloronema, which is made up of 6-8 short cylindrical cells, and the other portion is the rhizoids which germinated from the filamentous chloronema. There seems to be no primary rhizoid formed. The initial cell of the leafy gametophyte is formed at the base of the filamentous chloronema.

Peculiarity of the protonemal development

In *L. brachyptera*, green filamentous chloronema is first produced during the spore germination. The chloronema have numerous ovoid chloroplasts, and its cross-walls are at right-angles to the long axis of the protonemal filament. When the filamentous chloronema grows to a certain length, it continuously produces rhizoid, whose cross-walls are oblique. Meanwhile, the amount of chloroplast in the rhizoidal cells dramatically decreases to none. Rhizoid which does not contain any chloroplast is differentiated either from the base of gametophytes or filamentous chloronema. The slight and non-branched rhizoid grows in the inner side of the substrate. The growth rate of filamentous chloronema is slowed down when a gametophyte originated at the base of filamentous chloronema. However, the cells of filamentous chloronema obviously become stumpy and the green color becomes deeper.

Type of sporeling of *Lindbergia brachyptera*

The general characters of the protonema of *L. brachyptera* are: (1) spore germination takes place outside the spore wall; (2) the main element of protonema is filamentous, and the chloronema consists of short cylindrical or semi-globose cells; (3) the protonema system shows a tendency to have short branches with 5-10 cells, which occur at the base of filamentous chloronema. By referring to the classification of sporeling types published by Nishida (1978), the sporeling of *Lindbergia brachypter* belongs to the *Macromitrium*-type.

However, it differs remarkably from the *Macromitrium*-type in the following points: (1) the terminal part of chloronema differentiates into rhizoid, not into caulonema; (2) some filamentous chloronema cells have oblique wall but no chloroplast, making them look very similar to the rhizoid; (3) some filamentous chloronema cells are cross walled, but chloroplast is not found in them, either.

Because of the peculiarity of the protonema system of *L. brachyptera*, which is different from other mosses of *Macromitrium*-type, the sporeling of *Lindbergia brachyptera* needs further research.

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