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RESEARCH ARTICLE

Characteristics of spore germination and protonemal development in *Hypnum pacleseens*

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Abstract The spore germination, protonemal development, and gametophyte differentiation of *Hypnum pacleseens* were observed in cultivation. Photomicrographs showed that spore germination of *Hypnum pacleseens* occured within the exospore. Its protonema is massive with filamentous chloronema formed inside. The terminal part of the chloronema differentiated into filamentous caulonema and its rhizoid was derived from the apical cell of the filamentous chloronema. The initial cell of gametophyte differentiated from chloronema and caulonema. Sporeling-type of *Hypnum pacleseens* is developmentally similar to *Glyphmitrium-type*.

Keywords mosses, *Hypnum pacleseens*, spore germination, protonemal development, sporeling-type

1 Introduction

The gametophyte of mosses is haploid with a relatively simple structure. The life cycle of the moss starts from the germination of the spore to the development of protonema in contrast to the patterns exhibited by other plant groups. Since protonemal development in mosses can be analyzed in detail in living cells, they are excellent tools to study plant development at cellular and molecular levels.

Many investigations have been made particularly on morphological and physiological aspects of protonemal development of mosses since Hedwig first illustrated the protonema in 1782. Hofmeister was the first to systematically characterize protonemal development since records have been made. His observations showed that the sporeling of *Sphagnum acutifolium* consists of three elements: short chloronema, the rhizoid, and thallose protonema. In 1959, Nishida began extensive research on protonemal development and in 1978 she was able to develop a system to describe the sporeling-type of various mosses. In her doctoral dissertation, "Studies on the Sporeling Types in Mosses", she documented the 13 sporeling-types by the extensive analysis of 121 species (Nishida, 1978).

Currently, only afew doing research of moss development in China. Gao and Zhang studied spore germination and protonemal development of 9 species of Bryidae from China in 1986, 7 of which have never been reported before (Gao and Zhang, 1986). Bao et al. gave the detailed descriptions on the transition from spore germination to mature gametophyte of *Sphagnum* and *Marchantia polymorpha* (Bao and Cao, 2000; Bao and Cao, 2001). In 2002, Zhao et al. reported the spore germination and protonemal development of 13 species of mosses, such as *Physcomitrium eurystomum*, *Entodon maceropdus*, *Pylaisiella polyantha*, *Encalypta ciliata*, *Bryum uliginosum*, 12 of which were previously unidentified (Zhao et al., 2002; Fan et al., 2004; Zhao et al., 2004; Li et al., 2005a; Li et al., 2005b). Zhao et al. (2005) also analyzed the developmental characteristics of the gemma of *Barbula indica* and showed that it can assume unicellular and multicellular forms. The gemma can form not owly on the gametophyte but also on the protonema, and then the protonema can actually differentiate into a gametophyte. These features are adaptations to the dry environmental condition.

We hypothesized the potential mechanisms of spore germination and protonemal development of *Hypnum pacleseens* by comparing its developmental characteristics to other well characterized mosses. By studying the developmental characteristics of the *H. pacleseens*, we confirmed its sporeling-type and determined how its protonema develops. This study is important since it can aid greatly in clarifying the phylogenetic position of *H. pacleseens* among mosses and it can also contribute new knowledge to the reproductive

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characteristics of mosses. Zhang et al. (2002) suggested that mosses constitute the dominant portion of desert crusts and they also play an important role in increasing the thickness of biological crusts by modulating nutrient accumulation and dynamic equilibrium of moisture. Additional insights on the reproductive characteristics of the drought-resistant mosses were also revealed by studying the developmental characteristics of *H. pacleseen.*

2 Materials and methods

2.1 Experimental materials

Samples were taken from the mature capsules of *H. pacleseens*, voucher specimen: Fan Qingshu 030044 (HBNU), taken on October 11, 2003. *H. pacleseens* often grow on the rotten wood, bark, base of the trunk, withered branches and deciduous leaves beneath deciduous pinewood, birch, Mongolian oak and spruce forests. It can be found in north of Xingan (NGK), in the mountains of Helan (HL), and forest zones in the provinces of Heilongjiang, Jilin, Liaoning, Hebei, Shanxi, Sichuan, Yunnan, Tibet Autonomous Region, etc and *H. pacleseens* is also distributed in Japan, Korea, Russia, Europe and North America (Zhao et al., 2005).

2.2 Methods

2.2.1 The agar substrate used in our experiments contains the modified Knop's solution (Table 1). The agar powder used in this study was from Sigma Company.

Table 1 Modified recipe of Knop's solution

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
$NaNO_3$	minute
Distilled water	1L

Note: pH=7.0

2.2.2 Procedures

1) Preparation of substrate: The Knop's solution was prepared according to Table 1, agar was added to the solution to a final concentration of 2%. The mixture was then autoclaved and poured into the 60 mm sterilize containers and was allowed to solidify.

2) Preparation of spore fluid: On the aseptic bench in the laboratory, mature capsules of *H. pacleseens* were dipped in 75% ethanol and rinsed 5 times with distilled water**.** Sterile nipper and a dissecting needle were used to open the capsules to extract the spores. Spores were then mixed with 10 ml distilled water to make the spore fluid.

3) Inoculation of spore fluid: The spore fluid was transferred to the solidified agar substrate using a pipettor.

4) Incubation of spores: The samples were incubated in the RXZ controlled growth chamber under these parameters: 20 ± 2ºC; relative humidity of more than 80%, illumination intensity of 24 $lux\cdot m^{-2}\cdot s^{-1}$ at 12 hour light-dark cycle.

5) Inspection of spore germination: The growth conditions of the spores were observed daily. Microphotographs were taken as needed to illustrate germination of moss spores. Spore germination was observed under 10 × 10 microscopic eyepieces. In the course of the experiment, germinating spores were randomly selected for observation. Percentage of germinating spores were calculated by averaging the values obtained during 3 observations points.

3 Results

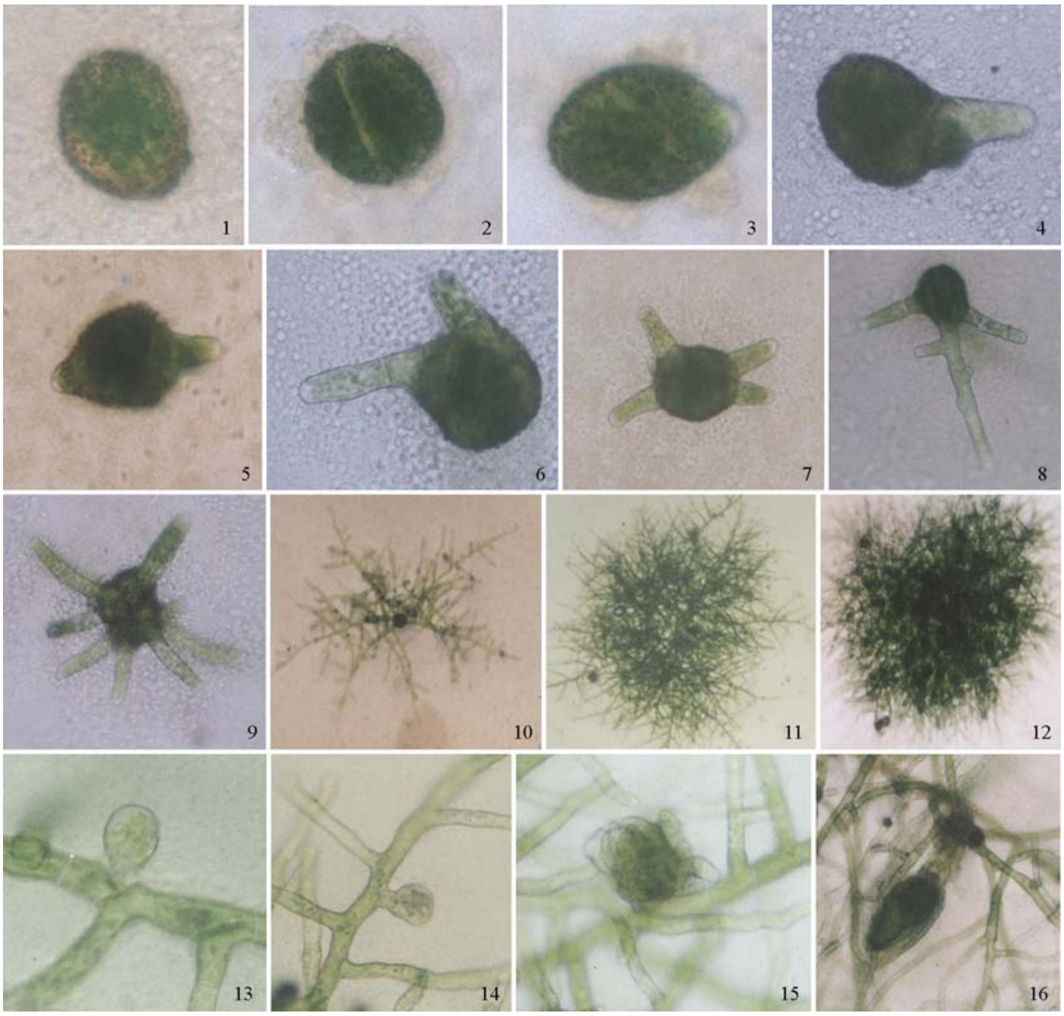
The spore of *H. pacleseens* is unicellular and germinates endosporously. It is spherical, pachypleurous, green in colour, and about 35μm (20~40μm) in diameter. A large number of mammillae were observed on the sporoderm which is composed of both exine and intine. Some undeveloped chloroplasts can also be seen inside (Fig. I:1).

Observations made under compound light microscope indicated that the process of spore germination to gametophyte formation has three distinct stages: the spore germination, protonemal development, and differentiation of the gametophyte.

3.1 Spore germination

The spore germination of *H. pacleseens* was observed to be divided into two consequential stages: (1) the spore absorbed water and swelled, and the amount of chloroplasts present increased continuously and moved to the central part of the spore. (2) On the second day, spore began to germinate inside the exospore and produced two equal massive protonema cells (Fig. I:2). Then the plasma membrane of a cell broke through the exine and began to project outside the spore and continued elongating, forming the initial cell of protonema (Fig. I:3,4). Meanwhile, another cell began to break through the exine and also formed the initial cell of protonema (Fig. I:5). The initial cell of protonema during extension also produced two cells (Fig. I:6), as it continued dividing, it formed a massive protonema containing 4~6 cells. Each cell differentiated into the initial cell of chloronema and by this time the average diameter of the massive protonema was about 53 µm.

Statistics of germination rate was taken on the 3rd day. The results showed 40% of the spores germinated. On the



1: Spore; 2: Spore showing the first cell division within the exospore; 3, 4: The initial cell of protonema projecting and elongating outside the spore; 5: Spore with bi-polar germination; 6: The initial cell of protonema producing new cells; 7, 9: The germinating spore with polypolarity; 8: Filamentous chloronema differentiating into primary branches; 10, 11: The branches of chloronema crossing each other forming a mesh; 12: Large amount of caulonema forming at the basal cells of the chloronema; 13, 14: The gametophytic initial cell differentiating from the basal cells of the chloronema or the caulonema; 15: The gametophytic initial cell dividing into a mulberry structure containing many cells; 16: Young gametophyte with 3~5 spires. (1~6，13×40; 7~9, 14, 15×20; 10~12×4; 16 ×10)

Fig. I The different stages of spore germination and protonemal development of *Hypnum pacleseens*

4^{th} day, the polarity of spore germination was observed wherein 23.6 %, 12.7%, and 15.1% were unipolar, bipolar, and tripolar, respectively.

In this period, no primary rhizoid and caulonema were formed.

3.2 Protonemal development

On the 7^{th} day, the germination polarity of 60% of the massive protonema was one to six (Fig. I:7), and 20% of the chloronema formed on the massive protonema have grown into 1−4 cells, with the length of 170μm. The diameter and length of the chloronema cell were 12μm and 50μm, respectively. On the 8^{th} day, the primary branch differentiated from the basal cell of the chloronema (Fig. I:8). At this time, more than 50% of massive protonema grew in one to eight poles (Fig. I:9) and the length of chloronema reached roughly 200 μm. On the 10^{th} day, chloronema further increased its size to 350 μm and its terminal cells began to differentiate into caulonema with oblique cross walls.

On the 14^{th} day, the branch of chloronema increased drastically and crossed each other to form dense mesh (Fig. I:10,11). Most of the primary branches present at the basal cells of the chloronema grew crookedly. The secondary branches with oblique cross walls derived from caulonema increased in number too, at this point the protonemal system started to project itself radially and that all apical cells of chloronema differentiated into caulonema. The length and diameter of the basal cells of the chloronema were 30μm and 10μm, respectively. The tertiary branches also increased in number, some were burrowed in the culture medium while others were projected outside. On the 20^{th} day, a large amount of caulonema formed and began to overlap each other especially those developing at the basal cells of the chloronema (Fig. I:12).

Secondary rhizoid also started to form at the terminal cell or branches of the chloronema .

3. 3 Gametophyte differentiation

On the 24th day, the initial gametophyte cell started to form next to the basal cells of the chloronema or on the caulonema itself (Fig. I:13, 14). At first, a single swollen cell protrusion formed in the chloronema. Five hours later, it started to divide and assume a mulberry structure containing many cells (Fig. I:15).Then asymmetric cell division commenced where in the cells divided at random planes gave rise to a three-dimensional structure. The cells even gave rise to meristematic cells that stretched outside the culture medium. Meanwhile, some white filamentous rhizoids started to differentiate at the basal cells of the gametophyte and their growth was influenced by positive geotropism.

On the 26th day, young gametophytes kept increasing in number, 80% of which can be found in primary and secondary branches. A great majority of the newly formed gametophytes can be seen in primary branches while the rest are distributed on other branches. As this event was taking place, approximately 10% of the caulonema turned brown. On the 28th day, one to three spires appeared on the apical portion of the mulberry-like meristematic cells. A large number of colorless rhizoids formed at the base of the young gametophytes. The rhizoids were long and thin, with oblique cross wall and devoid of chloroplasts.The gametophytes continued dividing into 3~5 spires in the following days (Fig. I:16). On the 35th day, up to 5~7 thalamic leaves can be seen to sprout from the young gametophytes.

4 Discussion

4.1 The particularity of spore germination and protonemal development of *H. pacleseens*

The defining characteristics of spore germination and protonemal development of *H. pacleseens* are germination within the exospore, massive multicellular protonema, and the initial cell of chloronema was derived from the edges of the massive protenoma.

The massive multicellular protonema can project at many poles at the same time and some can have as many as ten poles. The chloroplasts of chloronema cells of *H. pacleseens* are fewer than other mosses. Another remarkable characteristic of *H. pacleseens* is that its chloronema can differentiate into caulonema during the course of its development and most of its branches were derived from caulonema. The caulonema accounted for 70% of the whole protonemal system and it can grew crookedly during development. The whole protonemal system projected itself radial and the gametophytes could occur on both chloronema and caulonema, although, a great majority were formed on the caulonema.

4.2 Sporeling type of *H. pacleseens*

Spore germination of *H. pacleseens* occured within the exospore and the first wall separated the swollen spore into two cells of the equal size. It continued dividing for once or twice and formed a massive protonema containing 4~6 cells. Filamentous chloronema with cylindrical cell can also be seen on the massive protonema. There were two kinds of chloronema, one burrowed in the agar substrate and the other projected outside. The caulonema and the secondary rhizoid were all produced on the chloronema and the initial cells of gametophyte were formed on the chloronema or on the caulonema. These developmental characteristics and sporeling-type of *H. pacleseens* are remarkably similar to those of *Glyphmitrium-type*. However, unlike in *Glyphmitrium-type, H. pacleseens* have irregular cylindrical protonema cells and the caulonema can differentiate into a gametophytes. Thus they can be regarded as the new characters to supplement this sporeling type of *Glyphmitrium-type.*

4.3 Correlation between developmental characters and ecology adaptation

At the beginning of the *H. pacleseens* germination, the protoplast is divided within the exospore, forming massive multicellular protonema that do not develop into a filamentous protonemal system. Since *H. pacleseens* grow mainly on the trunk surface, its growth environment is more arid than the ground. Its non-filamentous protonemal system reduces its surface area making it less susceptible to excessive evaporation and dessication but at the expense of having a thin chloronema with fewer chloroplasts All of these characteristics are obviously adaptive strategies by which mosses make use of their niche environments.

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References

Bao W. M. and Cao J. G., Spore germination and sexual reproduction in *Sphagnum.* Chinese Bull. Bot., 2001, 36(1): 8−9[包文美, 曹建国, 泥炭藓及其孢子萌发和有性生殖. 生物学通报, 2001, 36(1): 8−9]

Bao W. M. and Cao J. G., Sporophyte and spore germination in *Marchantia polymorpha.* Bull. Biol., 2000, 35(1): 17−19[包文美, 曹建国, 地钱的孢子体和孢子萌发, 生物学通报, 2000, 35(1): 17−19]

Fan Q. S., Zhao J. C., Huang S. L., Experimental observations on the gametophyte development of *Pylaisiella polyantha*. Journal of Wuhan Bot. Research, 2004, 22(2): 140–144[范庆书, 赵建成, 黄士良, 金灰藓 (*Pylaisiella polyantha*) 配子体发生的实验观察. 武汉植物学研究, 2004, 22(2):140–144]

Gao Q. and Zhang Y., A preliminary study on spore germination and protonema development of the moss in China. J. Wuhan Bot. Res., 1986, 4(2): 123–132 [高谦, 张钺, 中国藓类植物孢子萌发和原丝体发育的初步研究. 武汉植物学研究, 1986, 4(2): 123–132]

Li M., Fan Q. S., Huang S. L., Zhao J. C., Zhang Y. M., Experimental studies on the characteristics of protonema development in *Encalypta ciliata*. J. Wuhan Bot. Res., 2005a, 23(1): 58–62 [李敏, 范庆书, 黄士良, 赵建成, 张元明, 大帽藓(*Encalypta ciliata*) 原丝体发育特征的实验研究. 武汉植物学研究, 2005a, 23(1):58–62]

Li M., Zhao J. C., Huang S. L., Fan Q. S., Zhang Y. M., Studies on the characteristics of protonema development of *Bryum uliginosum*. Acta Bot. Boreal. Occident. Sin., 2005b, 25(2): 363–367 [李敏, 赵建成, 黄士良, 范庆书, 张元明, 垂蒴真藓(*Bryum uliginosum*)原丝体发育特征的研究. 西北植物学报, 2005b, 25(2):363–367]

Nishida Y., Studies on the sporeling types in bryophytes. J. Hattori Bot. Lab., 1978, 44: 371–454

Zhang P., Bai X. L., Xu J., Wang X. D., Yong S. P., Jia X. H., Study on characteristic of moss reproductive biology in fixed dune soil crust of Shapotou Region. J. Desert Res., 2002, 22(6): 553–558 [张萍，白学良，徐杰，王先道，雍世鹏，贾晓红，沙坡头固定沙丘结皮层藓类植物繁殖生物学特性研究. 中国沙漠，2002, 22(6): 553–558]

Zhao J. C., Huang S. L., Li M., Mamtimin S., He J., Zhang Y. M., Li X., A study on the characteristics of spore germination and protonemal development in *Lindbergia Brachyptera*. Arctoa, 2004, 13:223–228

Zhao J. C., Huang S. L., Li M., Zang Y. M., Wang X. R., Fan Q. S., Experimental studies on the characteristics of gemma development in *Barbula indica.* Bull. Bot. Res., 2005, 25(2): 169–172 [赵建成, 黄士良, 李敏, 张元明, 王晓蕊, 范庆书, 小扭口藓(*Barbula indica*)芽孢发育特征的实验研究. 植物研究, 2005, 25(2): 169–172]

Zhao J. C., Li X. Q., Zhang H. Z., A preliminary study on spore germination and protonema development of ten species of bryophytes. Arid Zone Research, 2002, 19(1): 32–38[赵建成, 李秀芹, 张慧中, 10 种藓类植物孢子萌发和原丝体发育的初步研究. 干旱区研究, 2002,19(1): 32–38]