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Systematic Studies on the Conducting Tissue of the Gametophyte in Musci

(13) Anatomy of the Stem through Analysis of Pigment Deposition in *Polytrichum commune* HEDW. and *Pogonatum contortum* (BRID.) LESQ.

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Abstract Experiments on the absorption of pigments by the gametophytes of *Polytrichum commune* HEDW. and *Pogonatum contortum* (BRID.) LESQ. in which each tissue within their stems was dyed with different colors, led to the following three methods of dying being adopted.

1) Aniline blue-Eosin-Methyl green-method.
2) Janus green-Eosin-Methyl green-method.
3) Gentian violet-Congo red -Eosin-Methyl green-method.

By these methods, the cell walls of the epidermis were dyed brown-red by eosin and methyl green, the cell walls of the cortex were dyed blue-green by methyl green, and the chloroplast of the cortex were dyed red by eosin. The cytoplasm of the leptome was dyed red by eosin. The cell walls of the hadrome were dyed violet by the respective solutions of aniline blue and eosin, Janus green and eosin, and gentian violet and Congo red.

The relationship among gametophytes of the Musci was investigated according to the color adopted by each tissue, those showing similar dying properties being considered to be homologous tissues..

Introduction

The inner structure of the gametophyte in Musci, especially in mosses of the family Polytrichaceae, contains specialized conducting tissue which is necessary in land plants. HÉBANT (1977) has made an analysis of the inner structure of the stem in the Polytrichaceae and compared it with the stems of vascular plants. Also, TRACHTENBERG and ZAMSKI (1978) have given details of the hadrome and the leptome of the stem as a result of physiological experiments carried out on the gametophyte in the Polytrichaceae. It is not

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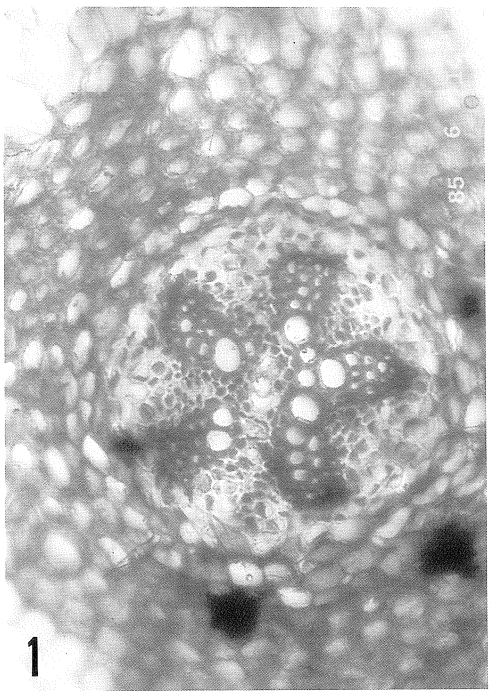
easy to understand the inner structure of the stem in the Polytrichaceae. It therefore seemed worthwhile to investigate whether each tissue of the stem in Musci is capable of being dyed with different colors, through the absorption of piments by the gametophyte.

We carried out a series of initial investigations using Congo red, Janus green, eosin, methyl green, eosin blue, methyl blue, fuchsin, gentian violet, thionin and aniline blue, containing over thirty pigments, in which various combinations of stains were used to produce different colors, and the results obtained indicated the suitability of the following three methods :

1) Aniline blue-Eosin-Methyl green-method
2) Jauns green-Eosin-Methyl green-method
3) Gentian violet-Congo red-Eosin-Methyl green-method

Using these methods it was shown that in vascular plants the cytoplasm of the phloem is dyed red by eosin, and that the cell walls of xylem vessels are dyed violet by the respective combinations of Janus green and eosin, aniline blue and eosin, and Congo red and gentian violet (Figs 1, 2).

In conformity with these results, the inner structures of stems of menbers of the Polytrichaceae were investigated.



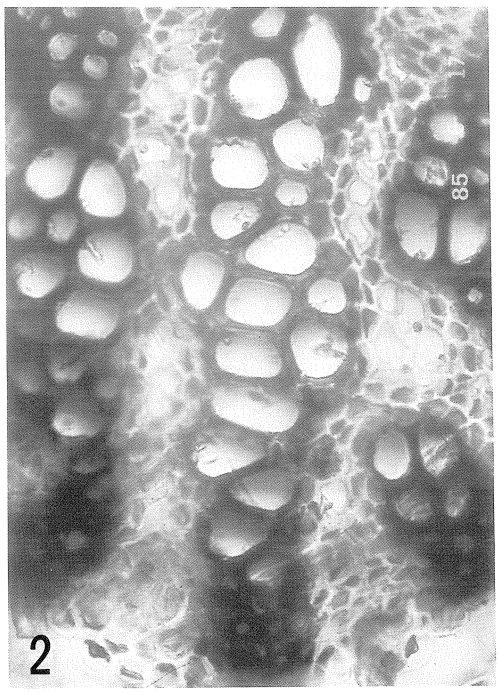


Fig. 1(x60) and Fig. 2(x200) : In the stem of *Lycopodium clavatum* L., the cell walls of xylem vessels are dyed violet with solution of aniline blue and eosin, and the cytoplasm of the phloem are dyed red by eosin solution.

Materials and Methods

The materials used for this research were specimens of *Polytrichum commune* HEDW. and *Pogonatum contortum* (BRID.) LESQ. of the Polytrichaceae seem to be the most differentiated of all the Musci.

Observations were conducted after subjecting the specimens to the following staining procedures.

Aniline blue-Eosin-Methyl green Method

(1) *Polytrichum commune* HEDW.

a) A solution of aniline blue and eosin was absorbed into the gametophyte for forty-eight hours.

b) After washing, a solution of eosin was absorbed for a further forty-eight hours.

c) After washing again, a solution of methyl green was absorbed into the specimen, again forty-eight hours. After washing cross-sections of the moss about 15μ in thickness were cut with a cryo-microtome and mounted in gum arabic.

(2) *Pogonatum contortum* (BRID.) LESQ.

a) A solution of aniline blue and eosin was absorbed into the gametophyte for thirty-two hours.

b) After washing, a solution of eosin was absorbed for a further forty hours.

c) After washing again, a solution of methyl green was absorbed into the specimen for thirty-two hours. After washing, cross-section of the moss about 15μ in thickness were cut with a cryo-microtome and mounted in gum arabic.

Janus green-Eosin-Methyl green Method

Polytrichum commune HEDW.

a) A solution of Janus green and eosin was absorbed into the gametophyte for forty-eight hours.

b) After washing, a solution of eosin was absorbed for a further forty-eight hours.

c) After washing again, a solution of methyl green was absorbed into the specimen for forty-eight hours. After washing, cross-sections of the moss about 15μ in thickness were cut with a cryo-microtome and mounted in gum arabic.

Gentian violet-Congo red-Eosin-Methyl green Method

Polytrichum commune HEDW.

a) A solution of gentian violet and Congo red was absorbed into the gametophyte for thirty-two hours.

b) After washing, a solution of eosin was absorbed for a further forty hours.

c) After washing again, a solution of methyl green was absorbed into the specimen for thirty-two hours. After washing, cross-sections of the moss about 15μ in thickness were cut with a cryo-microtome and mounted in gum arabic.

Results and Discussion

The order of absorption of pigments occurred in the same manner for the two species examined, but the period of absorption of pigments varied with species. Although these stems differed according to species, their homologous tissues showed identical staining properties, i. e.,

(1) The cell walls of the epidermis were dyed red by eosin solution..
(2) The cell walls of the cortex were dyed blue-green by methyl green solution and chloroplasts were dyed red by the eosin solution.
(3) The cytoplasm of the leptome were dyed red by the eosin solution.
(4) The cell walls of the hadrome were dyed violet by the respective solutions of aniline blue and eosin, Congo red and gentian violet, and Janus green and eosin.

The cell walls of the hadrome in the stems of Polytrichaceae are therefore dyed with solutions of aniline blue and eosin, Janus green and eosin, and Congo red and gentian violet. The cell walls of xylem vessels in the stem of *Lycopodium* are also dyed violet by in the same solutions. The cytoplasm of the leptome in the stems of Polytrichaceae are dyed red by eosin solution and it is interesting that the cytoplasm of the phloem in the stem of *Lycopodium* is also dyed red with eosin.

It may be that the leptome in Polytrichaceae and the phloem in vascular plants are homologous tissues, and that the hadrome in Polytrichaceae and the xylem in vascular plants are also homologous, as stated by HÉBANT (1977) and TRACHTENBERG and ZAMSKI (1978).

Further experiments on absorption and dying are currently in progress with the aim of investigating the relationship among the gametophytes of Musci.

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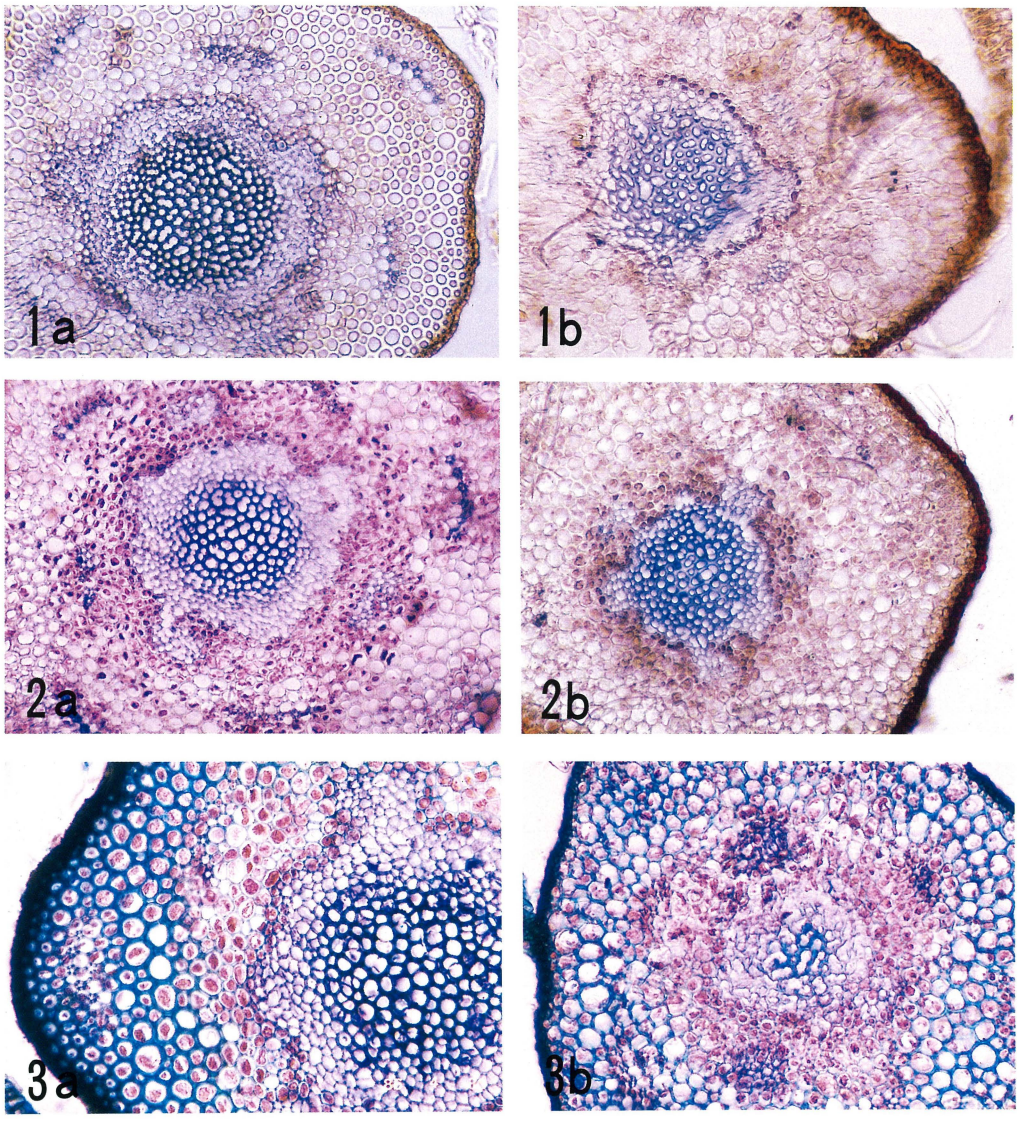

Plate I. Cross sections of the stem

Fig. 1a, 2a and 3a : *Polytrichum commune* HEDW.

Fig. 1b, 2b and 3b : *Pogonatum contortum* (BRID.) LESQ.

1) A solution of aniline blue and eosin was absorbed into the gametophyte for forty-eight (Fig. 1a) and thirty-two hours (Fig. 1b).

2) After washing, a solution of eosin was absorbed for a further forty-eight (Fig. 2a) forty hours (Fig. 2b).

3) After washing again, a solution of methyl green was absorbed for forty-eight (Fig. 3a) and thirty-two hours (Fig. 3b).

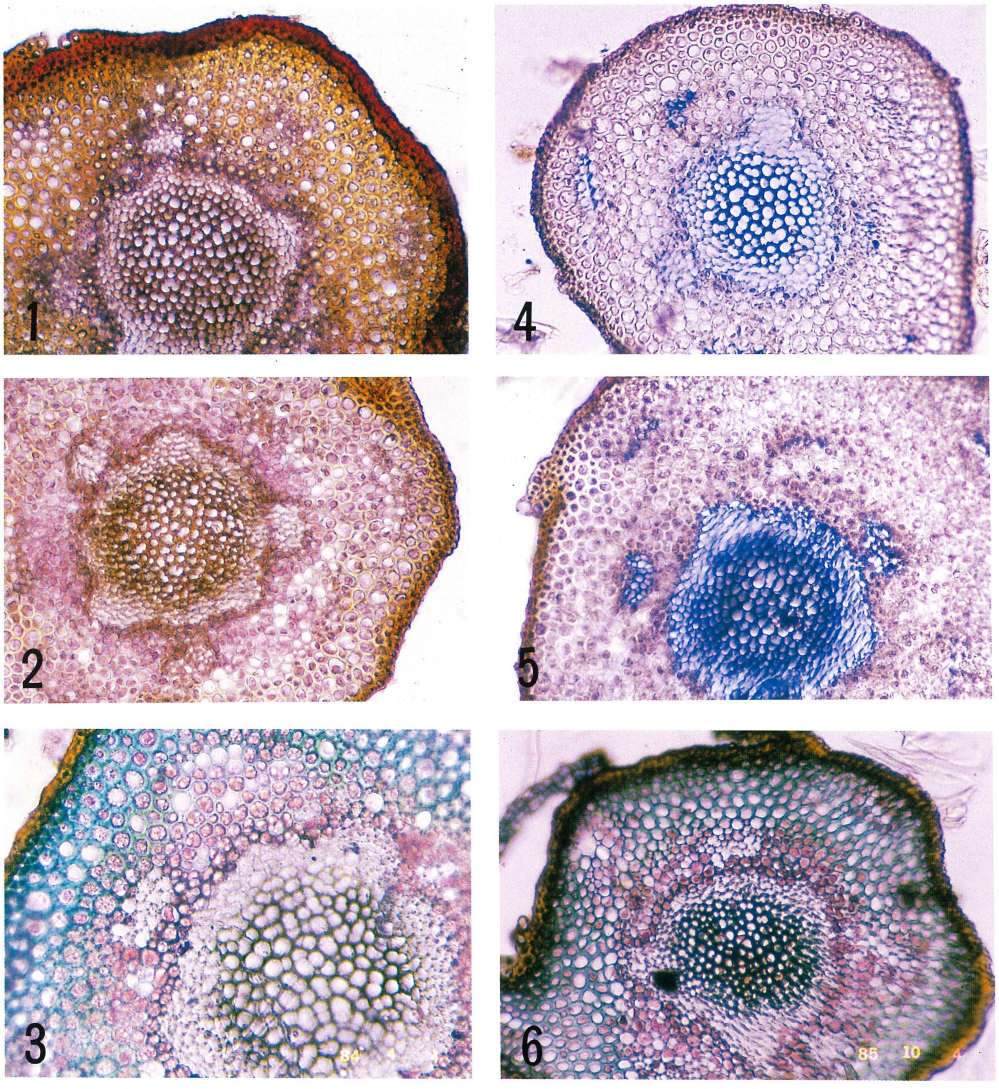

Plate II. Cross sections of the stem in *Polytrichum commune* HEDW.

1) A solution of Janus green and eosin was absorbed into the gametophyte for forty-eight hours (Fig. 1).
2) A solution of Congo red and gentian violet was absorbed into the gametophyte for thirty-two hours (Fig. 4).
3) After washing, a solution of eosin was absorbed for forty-eight hours (Fig. 2 and Fig. 5).
4) After washing again, a solution of methyl green was absorbed for forty-eight hours (Fig. 3 and Fig. 6).