GAMETOPHYTE DEVELOPMENT OF *TRICHOMANES SAXIFRAGOIDES*, PRESL.

by

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INTRODUCTION

This paper deals with the gametophyte development of an unfamiliar fern recently discovered by the writer in the vicinity of Taipei, Taiwan. The striking characteristics of the plant itself made obvious of its systematic position, belonging to the family Hymenophylaceae, genus *Trichomanes* and species *saxifragoides*, Presl[1].

In order to bring about a better understanding of the life cycle of *Trichomanes saxifragoides* and its phylogenetic relations which are not yet fully known, the writer has studied the stages in the development of its gametophyte based on sufficient materials collected and laboratory cultures of this newly found plant.

The obervations made thus far are presented here in this preliminary report. Other problems concerning the life cycle and phylogeny of *Trichomanes* Presl., in detail, will be treated thereafter.

MATERIALS

The materials used in this paper are based on a series of specimens collected from the vicinities of Taipei in Wu-lai, Shi-ting and Taipei, at four different times. Series I, inhabitated on a big moist rock near a creek, collected on May 10, 1951; series II, found at a dripping sandy cliff, in July, 1952; series III, at a shady wet cliff where several species of blue green algae and mosses entangled in the mass, collected on May 28, 1954; and series IV, at a sandy cliff, about 10 minutes walk eastward from Shi-ting, on June 2, 1954. All these areas are about 300 meters above the sea level.

DESCRIPTION AND DISCUSSION

Fascinated by the interesting appearance of *T. saxifragoides*, the writer has found the following features worthy of mention:

I. The minute sporophyte

Trichomanes saxifragoides grow in low patches, on moist shady grounds like a mass of Bryophytes (Fig. I). The sporophyte is minute as shown in figure II, 1. Its frond is one cell thick, about 5–8 mm. each way, dichotomous; sporangia (Fig. II, 3) stalkless, arising from a receptacle (Fig. II, 2); spores large and round, each about 57 μ in diameter; and annulus conspicuous, consisting of large cells with thick inner and radial walls. The stipe is slender, wiry and tomentose below; often more than one stipe arising at one spot from the slender rhizome (Fig. II, 1) which is creeping and

(1) Copeland, Phipi. Jour. Sci. Vol. LI, pp. 148.

interlaced.

II. The filamentous gametophyte

No description of the gametophyte of T. saxifragoides had ever been recorded in any literature which the writer came across. The facts enumerated below are the results of her own observations obtained from the study of the specimens and laboratory cultures as previously stated.

1. The germinating spores

When sporangia were crashed and examined under low power, a host of germinated spores, flooding out from the sporangium. Campbell[2] and Smith[3] declared that spores of this kind of fern germinated readily within the sporangia, which was a rather common phenomenon occurred in the members of *Trichomanes*. These spores resemble three-ray stars with new cells bulging out radially in three directions as in Fig. III, 1; each of the three cells grows but only one of them becomes the functioning filament, and the other two after dividing once or twice become less meristematic and finally stop dividing as shown in Fig. III, 2.

2. The gametophytic filament

The growing filament branches by enlarging its apical cell horizontally (Fig. VII, 1 and 2) and then it divides at both ends resulting a row of cells (Fig. VII, 3, 4). Consequently, it grows and increases in length to form the young gametophyte of T. saxifragoides. Tufts of them often crowd profusely around the mouth of the receptacle. These filaments resemble the protonema of moss, only they are visible to the unaided eye, and grow freely into the air. The cells in these gametophytic filaments are large, cylindrical and rich in chlorophyll. Rhizoidal cells in brown color arose on the filament, either laterally or terminally (Fig. III, 2 and 4) to perform the function of absorption.

3. The subsequent development of the gametophytic filaments

The further development of the gametophyte of T. saxifragoides is in a slow process. When germinated spores and young filaments were transplanted on agar medium, with a modified formula of Knop's solution[4] in flasks, very little growth took place. It was not until three or four months later that the filament began to shoot out from the receptacle and the longer ones attached spirally on the stipe. The germinating spores were transplanted in November and the above observations were obtained in April and May when growth showed most rapidly.

A closer examination of the new filaments under microscope revealed that the cells constituted were all alike, vegetative in nature. Definite sexual reproductive organs were not found. But besides the rhizoidal cells as mentioned before, there were a few swollen cells growing in pairs at the tips of some branches. Undoubtedly, they are the so called endophytic cells[5] containing an endophytic fungus as Fig. IV, 5 at the tips

(2) Campbell, Mosses and Ferns, pp. 360, 1895.
(3) Smith, G. M. Cryptogamic Botany Vol., pp. 317, 1938.
(4) Formula: Modified by Knudson, Knudson's sol. B.
(5) Smith, Cryptogamic Bot., pp. 300.

of two branches. Both Smith and Campbell[6] mentioned about some kind of mycorhizal fungus living in the rhizoids of certain filamentous gametophytes. This presence of a gametophytic filament and its slow development agree with what Bower, Campbell[7] and others had found in their work on Trichomanes.

4. The sex organs

Having found the gametophyte of Trichomanes saxifragoides the writer wondered whether these filaments would develop sex organs and produce sporophyte generation sexually as most of the ferns do. In order to answer that question she prepared a number of slide cultures for microscopic study. First, a few young filaments and germinating spores were thinly placed on clean slides with a drop of nutrient solution on each. Then the slides were numbered and placed in moist chambers. In every few days another drop of nutrient solution or water should be added so as to keep the cultures in constant moist conditions. In this way, the cultures could be kept growing for an indefinite length of time. It was from these slide cultures that the writer began to hunt for sexual differentiation in T. saxifragoides.

Bower (1888) pointed out that young sporophyte of certain species of Trichomanes might arise apogamously[8], that is, they were formed without the union of gametes. The writer, however, saw some kind of differentiation of vegetative cells occurred in some filament of her cultures. At first, there were two places rounding out like small bubbles at the conjunction of two adjoining cells (Fig. V, 1 and2). It occurred once before in an older culture but its development had not been followed up. On several other slides there were filaments bearing short-stalked, and much larger sac-like structures (with a diameter of 65 μ) (Fig. VI). Apparently, these were in mature state. Then four more of the same kind appeared successively on another filament of the same slide (Fig. VIII, 1). The contents inside of these seemed to have numerous protoplasts enclosed in a layer of transparent cells, somewhat resemble the antheridium of an ordinary fern, containing antherizoids. While the culture was being examined under high power, one of them, at the weight of a cover slip, broke open instantly, and out came the "presumed" gametes (?), with a drop of mucous substance dispersing into the surrounding water (Fig. VIII, 5). Figure VIII, 3 and 4 showed disintegrated and empty sacs with gametes already released. The approximate time required for its development from small bubble to full maturity was about three or four weeks in slide cultures. The supposed gametes when examined under high power, were transparent and oval or irregular in shape (Fig. VIII, 6). The number produced from each sac was about sixteen or more. After discharging of the gametes the empty sacs remained attached on the filament.

CONCLUSION

On the ground of the evidences as clarified in the foregoing, the fern treated above

(6) Campbell, Mosses and Ferns pp. 365.
(7) Campbell, pp. 361.
(8) Bower 1888 Ibis I: 269-305, 3 pl (Apogamy Trichomanes)

is Trichomanes saxifragoides Prel (Hooker Synopsis Fil. 1867, 76).

The gametophyte of T. saxifragoides is filamentous, branching profusely by fission and budding in addition to the lateral division of the apical cells when they are in young stages.

A differentiation of the vegetative cells of the filaments into gametangia-like structures has been observed. Since there were no other specializations present on the filaments other than the one mentioned, nor was there any difference of morphological features noticed among them, the substance produced from them might be regarded as isogametes or perhaps, they function like isogametes, in the process of reproduction. But whether there is any union of these isogametes or whether gametes act as spores to produce sporophyte of T. saxifragoides directly from each one of them, is difficult to tell at present. Further knowledge is necessary in order to have a complete understanding of the subject. However, this differentiation of vegetative cell may indicate an approach to the formation of certain advanced type of sex initiation. Further more, its filamentous gametophyte may also illustrate a close relationship to the persisting filamentous body of the ancestral algae.

Further study on this subject will be continued and it may produce interesting results.

IMPORTANT REFERENCES

(1) BOSCH, VAN DEN.: Hymen. Jav. pp. 8-9, Tab. III, Figs. 1, 3.
(2) CAMPBELL, D. H.: 1895. Mosses and Ferns, pp. 360-370.
(3) COPELAND, E. B.: 1933. Phili. Jour. Sci., Vol. LI, pp. 119-280.
(4) HOOKER: 1868. Synopsis Filicum. p. 75.
(5) HOOKER: Species Filicum, Vol. 1, p. 118, Tab 39 A
(6) SMITH, G. M.: 1933. Cryptogamic Botany, Vol. II, pp. 313-319.

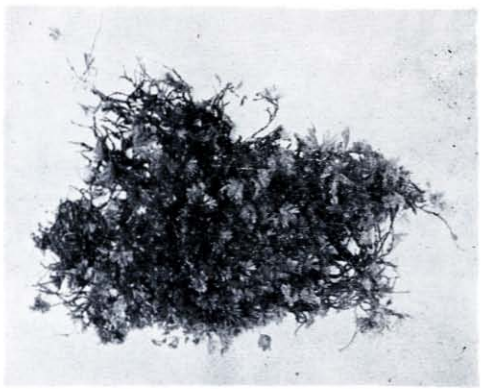

Fig. I. Trichomanes saxifragoides, Presl., growing in a patch. (Natural size)

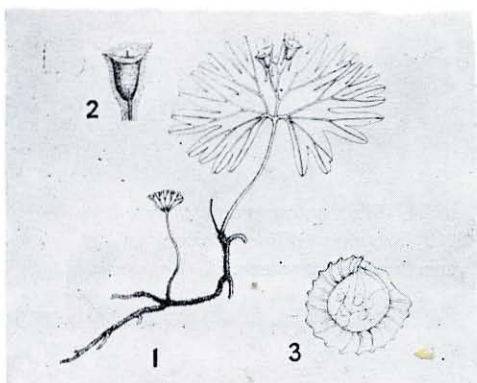

Fig. II. A Single sporophyte (15x)
1. Two fronds
2. A sorus (5x)
3. A sporangium (16x 10x)

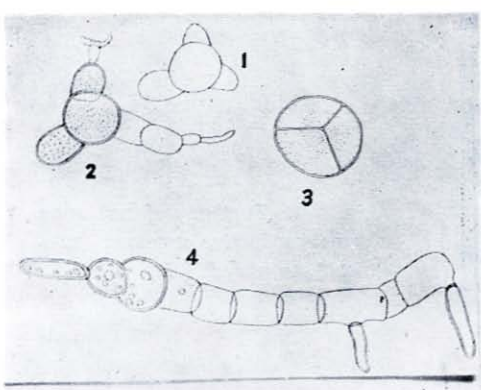

Fig. III. Germinating spores.
1. A spore with bulging rays.
2. A spore with a young growing filament
3. A tetrad
4. A full grown filament

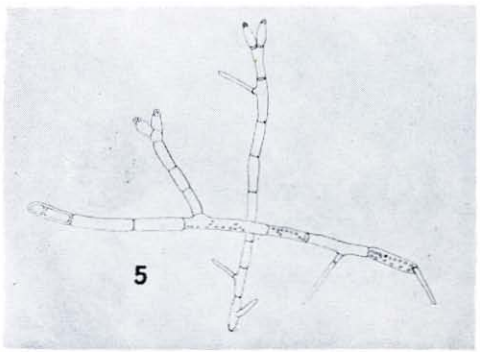

Fig. IV. Filamentous gametophyte with rhizoids and swollen endophytic cells at the tips of two branches.

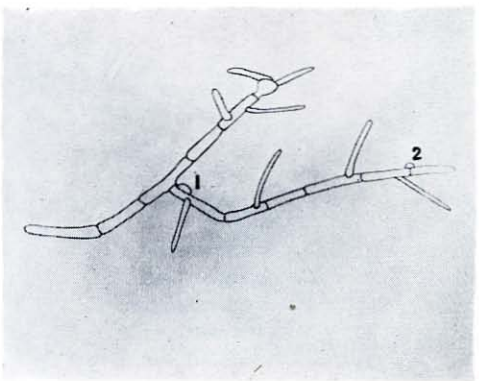

Fig. V. Filamentous gametophyte. 1, 2 showing differentiation of reproductive structure.

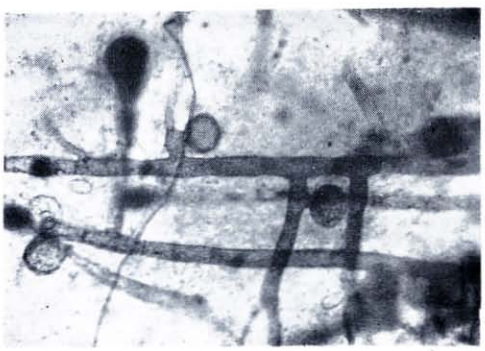

Fig. VI. Further differentiation of reproductive structure (microphoto under H. P.)

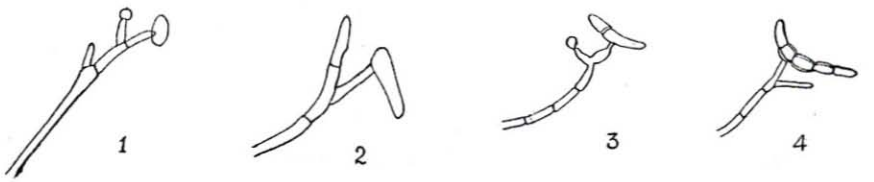

Fig. VII. Branching of gametophytic filaments. 1, 2. Enlargment of apical cells. 3, 4. A row of New cells resulted from successive dividions.

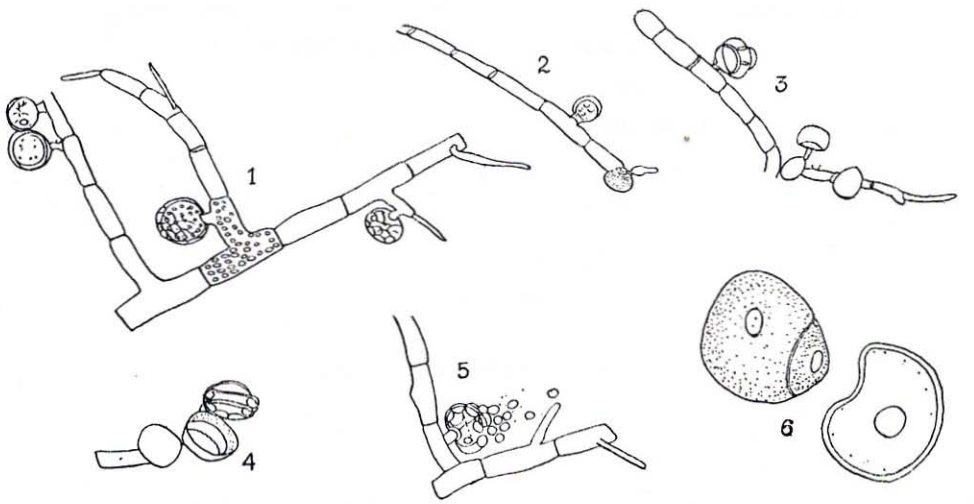

Fig. VIII. Different stages in the development of reproductive structures. 1. Mature 2. Young stage 3, 4. Empty ones, after contents discharged 5. Discharging of probable gametes 6. gametes, magnified.