A STUDY ON THE REGENERATION OF *PALLAVICINIA LONGISPINA*[1]

BAO-YU YANG[2]

INTRODUCTION

Earlier works on the family Pallaviciniaceae were confined to morphological and more frequently taxonomic treatments as represented by Campbell and Williams (1914), Cavers (1903), Evans (1937), Farmer (1894) and Haupt (1918, 1943); and the most recent paper on the subject was a study of 5 species of New Zealand Pallavicinia by Hodgson (1965). More than 40 species of Pallavicinia have been described by various workers. Smith (1966) gave the first report on the conducting system of *Pallavicinia lyellii* and *Symphyogyna circinata*.

This study summarizes the results gathered from observations on the regeneration of a Taiwan species, *Pallavicinia longispina*, and the effects of different media and different light sources upon the growth and the mode of branching of the thallus.

MATERIALS AND METHODS

I. Materials.

Collections of *Pallavicinia longispina* used in this study were made on September 5, 1968, from various areas along the slope cliffs on the way to Cho-shan (祝山), Ali shan in Chiayi County by Bao-yu Yang, Jen-rong Wang, M. T. Kao and 3 students: Misses S. M. Lee, Y. S. Yang and M. Ting.

Most of the materials were found growing in great patches, some were stipitate and others procumbent on the wet shaded cliffs or on the stone walls of caves.

II. Methods.

1. Maintenance of the culture:

After collection the materials were brought into the laboratory and kept in glass jars which were 5 cm tall and 9 cm in diameter, under controlled condition. The temperature was maintained at 20°C; the light intensity at between 1200–1500 lux and with 8 hours of light per day. Cultures under such artificial conditions were maintained in good condition for long periods and are ready for use at any time.

2. Media: Basal nutrient solution.

KNO_3 ..... 1.02 g.
$Ca(NO_3)_2 \cdot 4H_2O$ ..... 0.492g.
$NH_4H_2PO_4$ ..... 0.23 g.

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(2) Professor of Botany, National Taiwan University. (楊寶瑜)

H_3BO_3.....0.86mg.
$MnCl_2 \cdot 4H_2O$.....1.81mg.
$CuSO_4 \cdot 5H_2O$.....0.08mg.
$ZnSO_4 \cdot 7H_2O$.....0.22mg.
Distilled water.....2,000 cc.
Trace elements: $FeSO_4$, Tartaric acid.

3. The following tests were performed:
   (1). Test for regeneration:
   Cuttings of small pieces (3–5 mm. long) of the present thallus were planted on the surface of agar plates with basal nutrient solution+1% agar+0.25% activated charcoal.
   (2). Test for regeneration under different light sources:
   Two agar plates were prepared as in (1) and placed in the dark, 2 agar plates prepared as in (1) were placed under red light, and 2 agar plates prepared as in (1) were placed under a fluorescent light.
   (3). Test for regeneration with different growth media:
   Basal nutrient solution+agar 1%+activated charcoal 0.25%.
   Basal nutrient solution+1% Sucrose+1% agar+activated charcoal 0.25%.
   Basal nutrient solution+1% Glucose+Glycine (10.0 mg/20 ml)+1% agar +activated charcoal 0.25%.
   Basal nutrient solution+1% Glucose+Alanine (22.4 mg/20 ml)+agar 1% +activated charcoal 0.25%.
   Basal nutrient solution+1% Glucose+Proline (32.8 mg/20 ml)+agar 1% +activated charcoal 0.25%.
   Basal nutrient solution+IAA 10^{-6} M+agar 1%+activated charcoal 0.25%.

OBSERVATIONS AND RESULTS

1. Results on basal medium: The cuttings (3–5 mm long) from selected thalli gave rise to primary branches. Differentiation of the branches took place in about 18 days. One month later, the average length of the branches on one of the plates reached about 0.7 cm. These primary branches began to fork dichotomously and eventually extended all over the surface of the medium (Pl. I figs. 1–4). The lateral branches appeared profusely from the prostrate branches, and these later turned upward and became erect and were no longer prostrate. Consequently, these new thalli were now dendroid in form instead of being simply flat thallose plants.
   All the lateral branches were initiated ventrally from the cells of the midrib.
2. The effect of light on branching: No branches were formed when the plates were kept in the dark. Few branches developed under red light but they developed profusely under fluorescent light. One plant grown under fluorescent

light produced branches that were almost uniform in size and length (Plate IV, 1–5).

3. The newly developed branches when separated from the midrib of the parent thallus developed into new individual plants.
4. Cultures grown on basal medium+sucrose showed the differentiation of branches most rapidly and their growth was vigorous (Pl. II, 2). Each branch gave rise on the average to 4 secondary branches in 14 days, and these soon began an upward growth, turning away from the agar surface. In addition sex organs were differentiated, most of them being archegonia (Pl. III, 1–5).
5. Cultures planted on basal medium+amino acid were easily contaminated and became very moldy within 4 or 5 days, especially so when alanine or glycine was applied. 70 days later, however, branches appeared in all directions from the thalli when proline was added to the basal medium (Pl. II, 3, 5, 6). These showed as many as 25 secondary branches had developed, and most of them were prostrate and not ascending, as was found in the medium to which sucrose had been added.
6. When IAA 10^{-6} M was added to the basal medium, more vigorous growth and more numerous secondary branches were developed than when grown on basal medium alone (P. II, 4) or when grown on any media to which any of the amino acids had been added except proline.
7. The power of regeneration in *Pallavicinia longispina* is vividly shown in Pls. I, V, and VI. Growth and development of the thallus rests upon the activity of cells in the midrib rather than those in the wings. Plate V shows a sequence of development from the activity of single cells from the midrib, beginning with primordia of a few cells (Figs. 1–4), and gradually reaching the elongated branch-stage with mucilaginous papillae and secondary primordia developed on it (Figs. 5, 6).
8. The power of regeneration is further evidenced in Plate VI. When wings or parts of them were cut off or destroyed by mechanical injury, new branches developed readily (Figs. 1, 2, 3, 4).

DISCUSSION AND CONCLUSION

1. The power of regeneration in *Pallavicinia longispina* is very strong as is demonstrated in the rapid growth of the cuttings from a selected thallus into new branches which turned out to be new individuals, asexually produced.
2. Various experiments have shown the power of regeneration and that the growth is due to the activity of the cells in the midrib, either apical or otherwise. The wing cells do not show this power of regeneration, they perform the vegetative functions such as absorption, assimilation, and photosynthesis, but not regeneration. Cells around the center of the midrib (the conducting system) when richly

supplied with nutrients may grow faster than cells from other parts of the thallus. Whether every midrib cell is capable of performing growth or whether this power of growth is restricted to a few special cells, time has not permitted us to investigate in the present study.

3. The fact that when amino acids were added to the basal medium, serious contamination resulted, may be due to the fact that glucose was always a part of the medium and this is favorable to fungal growth. The cause for rapid stimulation of the growth and the production of numerous branches when proline was added to the medium is not yet known. Basal medium with sucrose caused an upright growth which may indicate an uneven distribution of the auxin content in the cells of the midrib. The occurrence of archegonia was also found in relation to the sucrose in the medium. IAA 10^{-6} M, when added to the basal nutrient solution makes a more favorable medium for the growth and development of *Pallavicinia longispina* than any other media.
4. Lastly, the effect of light source upon the growth and development of *Pallavicinia longispina* shows that fluorescent light gives the best results, while red light stimulates the formation of a few branches, darkness inhibits the formation of new branches in the culture.

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EXPLANATION OF FIGURES

Plate I

Regeneration observed in *Pallavicinia longispina*

1. Culture (Plate no. 5) developed from cuttings (3-5mm long) of a selected thallus, reaching a steady growth on basal medium in 6 months; showing prostrate, dichotomous branching (figure not shown here) at first, then later, lateral branching, producing evenly spaced branches as shown in the figure.

2, 3, 4. Enlargements of Figure 1.

Plate II

Effect of media on growth and development

1. Thalli grown on basal medium, showing normal growth.
2. Culture on basal medium+sucrose, showing vigorous growth and numerous new branches 18 days old.

3, 5, 6. Culture on basal medium+proline; showing profuse branching.

4. Culture on basal medium with IAA 10^{-6} M, showing vigorous growth and numerous branches.

Plate III

Occurrence of archegonia in cultures grown on basal medium+sucrose

1, 2. Showing clusters of archegonia developed along the midrib. × 1.5 × 6.3

3, 5. Archegonial cluster and involucre dissected in parts × 25.

4. Showing archegonia, involucre removed × 40.

Plate IV

Effect of light source upon the growth and development of *Pallavicinia longispina*

1, 2. Cultures grown under fluorescent light (1200-1500 lux) showing normal growth and branching.

3, 4. Cultures grown under red light, branches poorly developed.

5. Culture grown in dark showing no branches formed.

Plate V

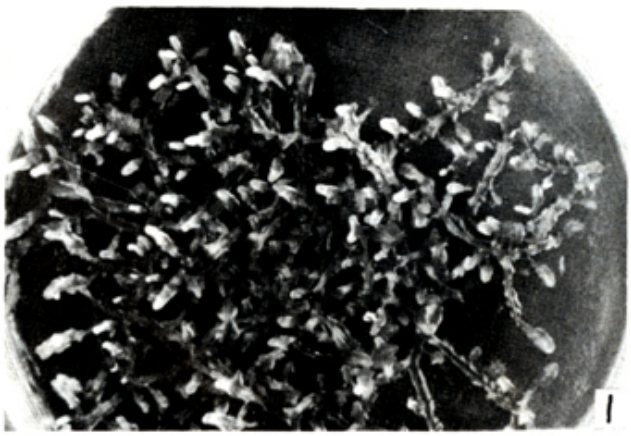
Power of regeneration as observed in *Pallavicinia longispina*

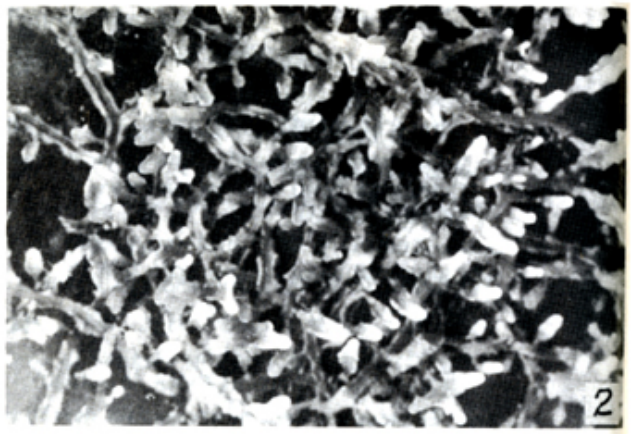
1, 2, 3, 4. Showing primordia of different stages initiated from cells of the midrib.

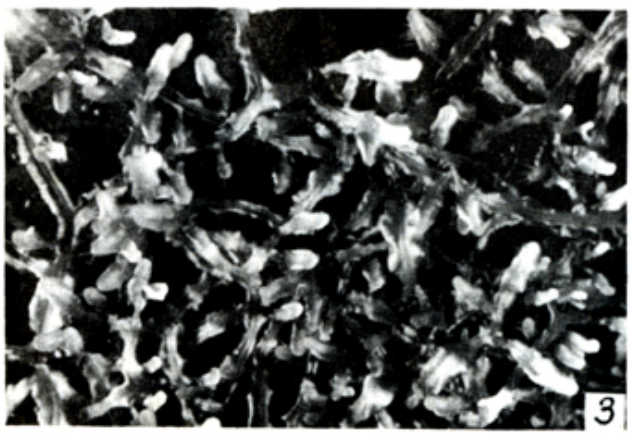
1. Primordia one or 2 days old × 70.
2. Primordia 3 days old × 70.
3. Primordia 5 days old × 70.
4. Papillae on 8 day old primordia × 70.
5, 6. Gradually reaching elongated branch-stage with mucilaginous papillae

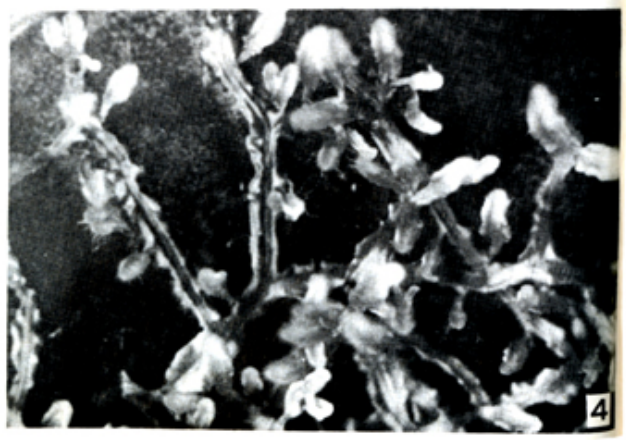
Plate VI

1. Branches arising from cells of a thallus with wings and midrib injuried.
2. Wings of thallus destroyed but new branch developing after a period of 3 or 4 months.
3. Wings destroyed by burning but midrib not disturbed, apical cell giving rise to a group of branches, showing dichotomy in some of them.
4. Primordia and new branches arising from a thallus with wings partly destroyed × 6.3









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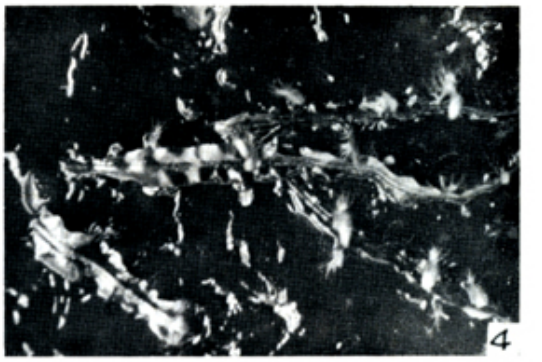

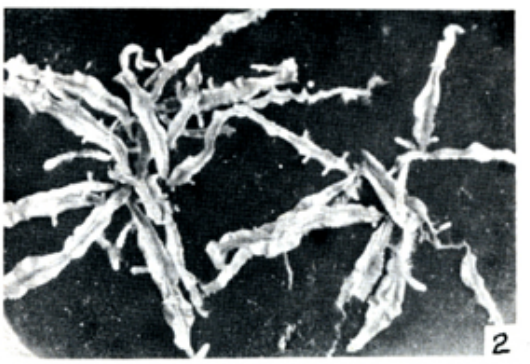

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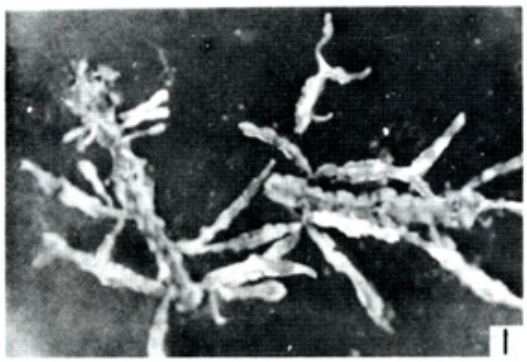

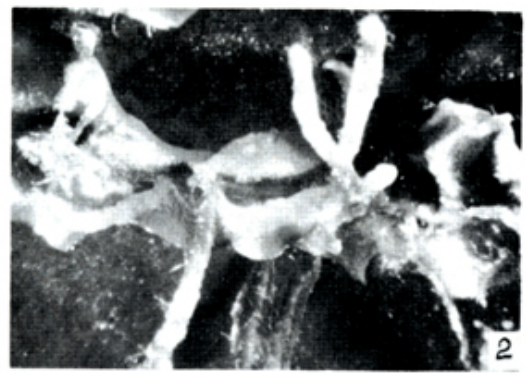

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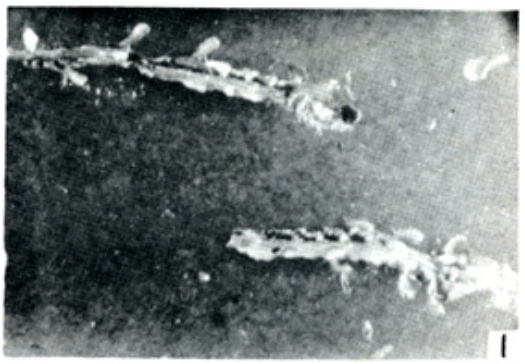

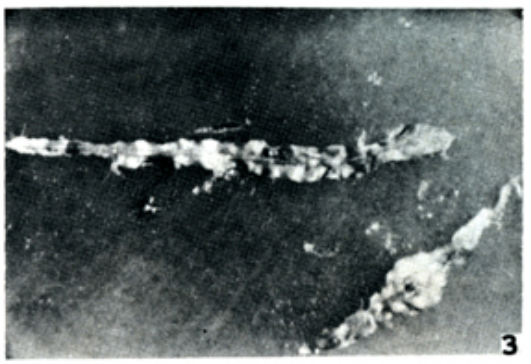

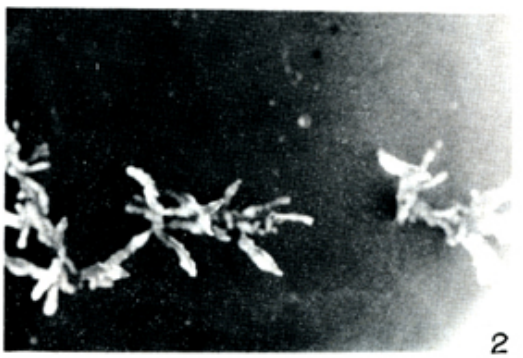

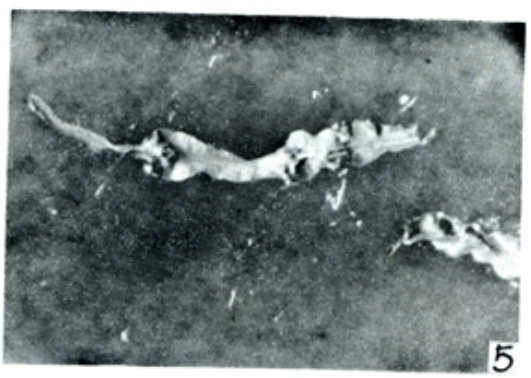

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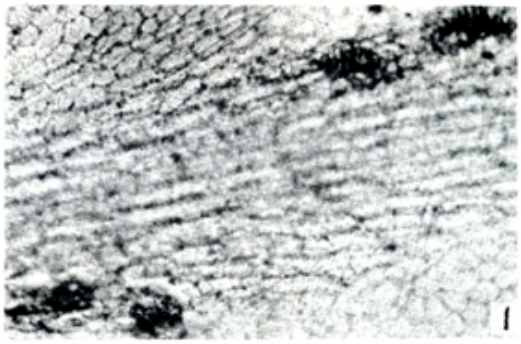

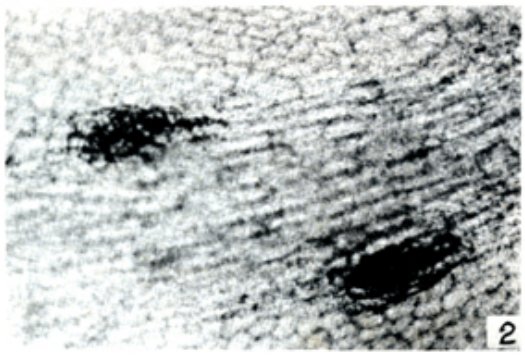

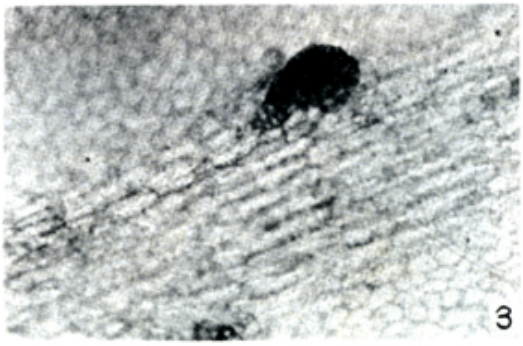

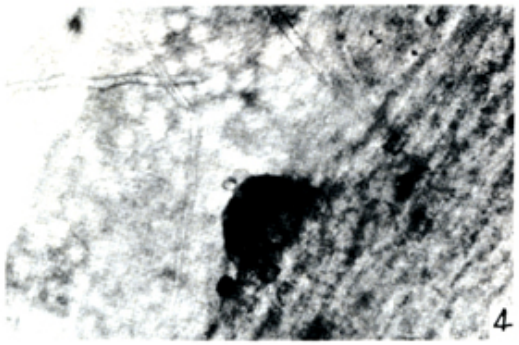

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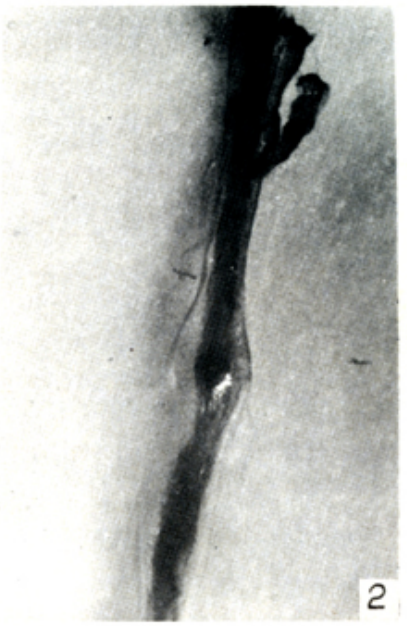

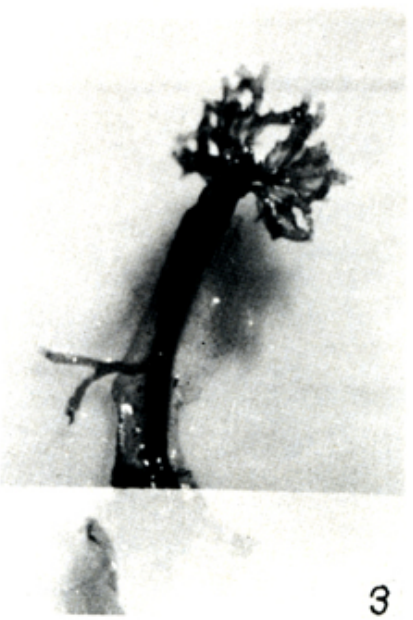

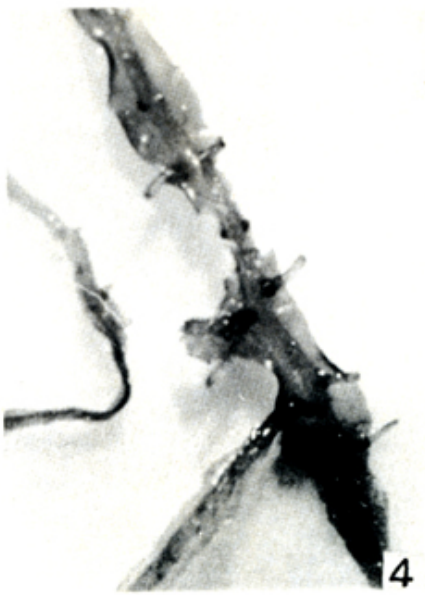

Plate VI