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Moss regeneration on the example of *Aulacomnium palustre* (Hedw.) Schwaegr

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Abstract

The developmental dynamics of *Aulacomnium palustre* populations finds its reflection in vegetative reproduction and regeneration. Leaves detached from the shoot form two kinds of protonemata — chloronema and caulonema, on which the gametophore develops. Most active in the process of regeneration is the basal part of the leaves. Increased light intensity speeds up regeneration.

INTRODUCTION

Reproduction by alternation of generations, that is a biphasic development cycle, considered generally as normal, plays a minor role in natural conditions than does vegetative reproduction and regeneration, particularly in species producing rarely sporophytes as for instance *Aulacomnium palustre*.

Investigations on leaf regeneration in the family *Polytrichaceae* were performed by Chopra and Sharma (1958) and in the family *Mniaceae* by Lersten (1961) and Misiura (1964).

The present paper deals with leaf regeneration in *Aulacomnium palustre*.

The *in vitro* cultures were grown under various edaphic and light conditions with the aim of accelerating and checking the processes occurring also in normal environment.

Vegetative reproduction and regeneration ensure the durability of moss populations many-years persistence, until the ecological factors change radicaly in the environment.

MATERIAL AND METHODS

The investigations were performed on the selected moss species *Aulacomnium palustre* collected on Łąki Sierakowskie (meadows) in the Kampinos National Park.

Observations on regeneration were carried out from December, 1972 to March, 1973 and from October, 1973 to January, 1974. From the moss weft individuals were isolated and carefuly washed in running tap water in order to remove soil contaminations. Then the green leaves were removed with pincers and washed in distilled water.

In the first experiments of December, 1972, 80—100 leaves were placed on Petri dishes (10 cm in diameter) with different substrate:

1. agar (25 g in 1000 ml of dist. H_2O) with Knop's medium, 1861). pH of the medium was 5.8. The medium was added in 5 and 10 ml amounts to 30 ml agar;
2. sand washed in tap water, then in distilled water and roasted (with Knop's medium — 5 and 10 ml)
3. peat.

The dishes were placed in aquaria with water at the bottom (to maintain a high moisture). The culture was illuminaed from above with fluorescent tubes of 360 W jointly for 10 h daily in the course of the whole experiment.

The second series of dishes was placed on the window sill.

On account of the changing substrate which dried up or was overgrown with algae, the leaves were transferred to dishes with distilled water, washed and placed on freshly prepared agar with nutrient medium. This procedure was repeated four times. The experiment was controlled at 7-day intervals in order to make observations on the stage of regeneration and record the sites at which the protonema grew out of the three main parts of the leaf: 1. the rib, 2. the basal part and 3. the medial part.

At each checking time 10 randomly chosen leaves from each dish were examined.

The next culture series in the second experiment with regenerating leaves was started in October, 1973 and lasted till January, 1974 on the following substrates:

1. agar with Knop's medium (20 ml in 1000 ml agar),
2. sand with 2 per cent of Knop's medium,
3. peat,
4. liquid 2 per cent Knop's medium.

The second experiment was run in a lumistat with joint illumination of 360 W. On account of changes in the agar substrate, like in the previous experiment, the leaves were transferred to fresh medium.

The culture was checked every 7 days by examining 10 randomly chosen leaves from each dish.

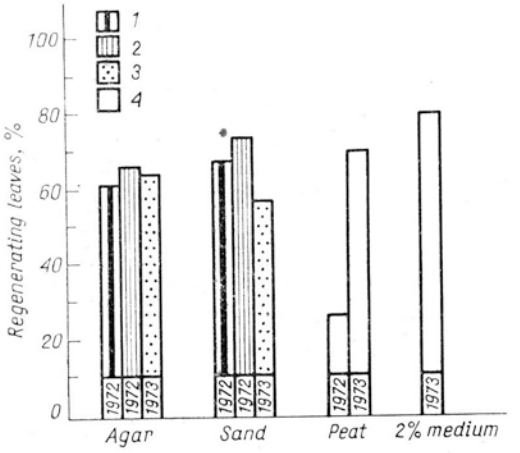


Fig. 1. Leaf regeneration (%) on various substrates under fluorescent tube illumination — 360 W (Experiment I)

Substrates: *1* — 5 ml Knop medium/30 ml agar; *2* — 10 ml Knop medium/30 ml agar; *3* — 30 ml 2%-medium; *4* — peat

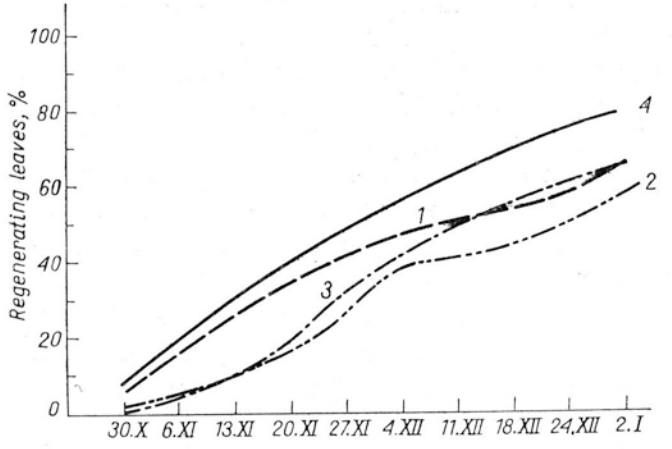


Fig. 2. Regeneration of leaves on various substrates: agar with 2% Knop medium (*1*), sand with 2% Knop medium (*2*), peat (*3*), liquid 2% Knop medium (*4*) at various periods of the experiment II

During the entire experiment 1600 leaves were examined, 400 from each substrate. The sites at which protonema grew out were recorded as in the first experiment.

Beside the above described experiments, microscopic observations were performed on material brought from the field and mass appearance of leaf-shaped gemmulae was noted.

RESULTS

Aulacomnium palustre reproduces vegetatively by flagella and leaf-shaped gemmulae which are a manifestation of leaf polymorphism (Berthier, 1972). Leaf-shaped gemmulae in natural conditions occur at the tips of shoots gathered in "heads". In culture they may be obtained during regeneration of flagella (Plate I, 2a, 2b, 2c, 2d). Protonemata grow from these gemmulae or directly buds giving new plants.

Beside special organs of vegetative reproduction, the gametophyte of *Aulacomnium palustre* has a high regeneration ability. In order to check the regenerative properties of this species two experiments were performed.

In the culture of the first experiment the influence of illumination conditions and edaphic factors on leaf regeneration was checked.

The results of this experiment (Table 1) indicate that the highest per cent of leaves regenerates on sand (75.0%) under fluorescent tube illumination and the lowest on peat under natural daylight (23.3%).

The results of regeneration of the particular parts of the leaf show that the basal part of the leaf is most, and the medial part least viable.

For testing the influence of the substrate on the regeneration ability a second experiment was performed. Various substrates were used and illumination of 360 W was constant. It appeared that the leaves regenerated best on Knop's liquid 80.0 per cent medium (Tables 1, 2). On sand and agar with 2 per cent Knop's medium added the result was similar to that in the previous experiment. On peat, however, the differences between the two results are controversial. In the first experiment the degree of regeneration was 26.6 per cent and in the second 65.0 per cent. The result of the second one is more convincing.

Regeneration depends on the vegetation period. The plant material in the second experiment in October exhibited a higher viability than that in the first experiment in December. The regeneration results on various substrates under the same illumination were similar.

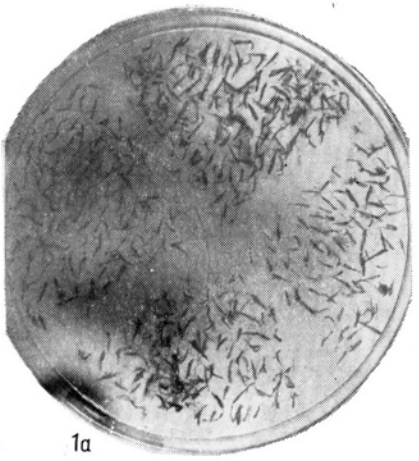
On sand and peat regeneration was retarded as compared with that on other substrates.

The dominating role of the basal parts of leaves in regeneration was confirmed in the second experiment.

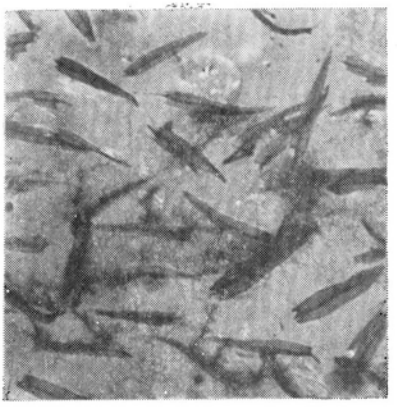
In leaves deteached from the shoot and transferred to various substrates a cell becomes active, mostly in the basal part, which dividing forms a protonema (Plate II, 1a, 1b, 1c) of the type of:

a) a chloronema characterized by thin cell walls and straight, colourless septa, numerous chloroplasts and a poorly visible nucleus (Plate II, 2b);

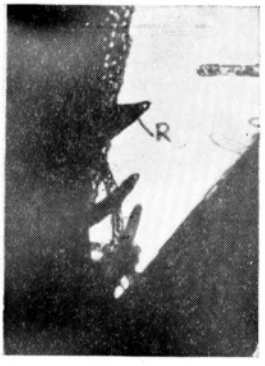
Plate I



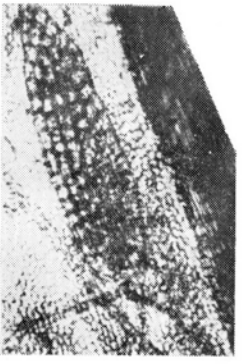
1a



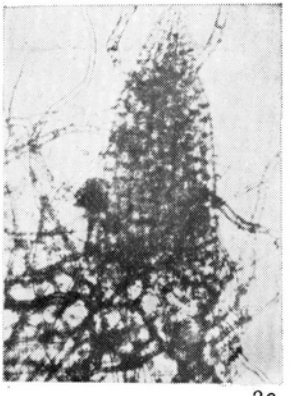
1b



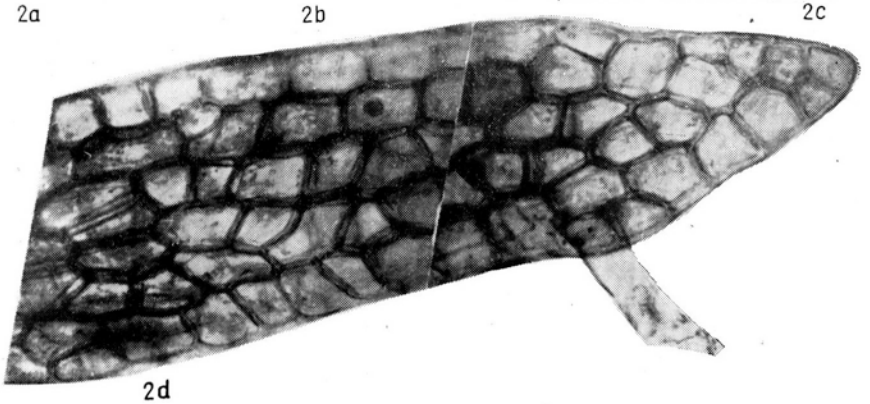
2a



2b



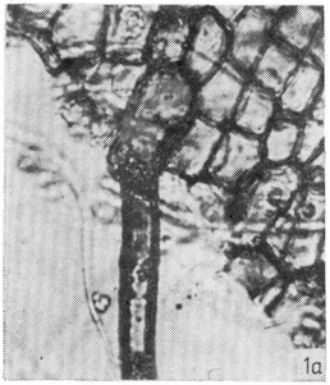
2c

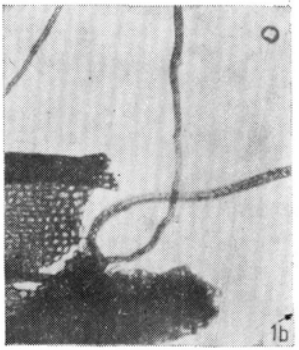


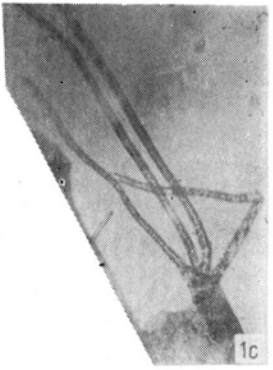
2d

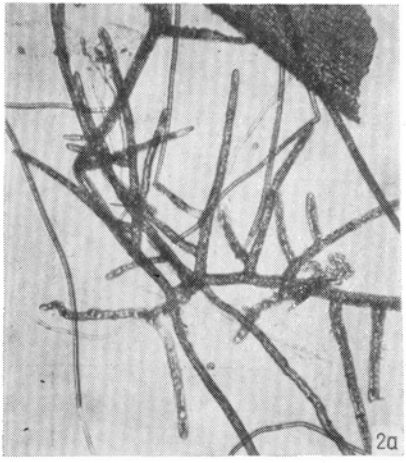
1. Leaf culture in Petri dishes: a) in agar with Knop medium added; b) regenerating leaves — branching protonemata are visible. 2. Leaf-shaped gemmulae: a) gemmulae in leaf axil R; b) gemmulae on leaf surface; c) gemmulae with growing out protonemata; d) fragment of cellular structure of gemmula

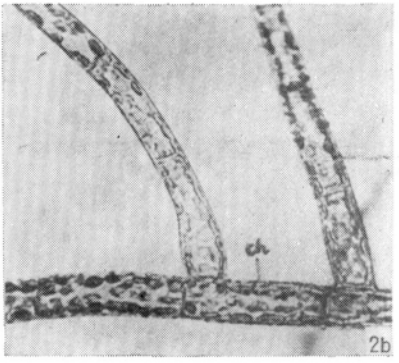
Plate II

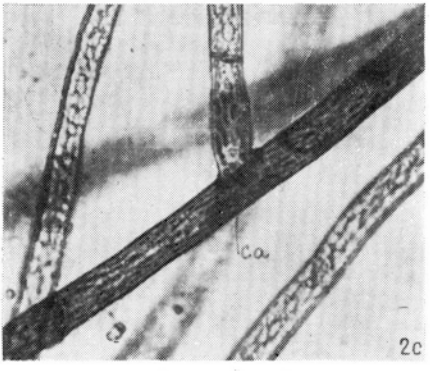


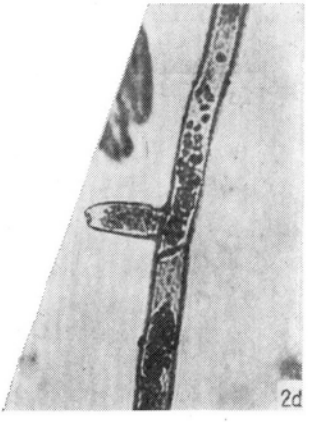






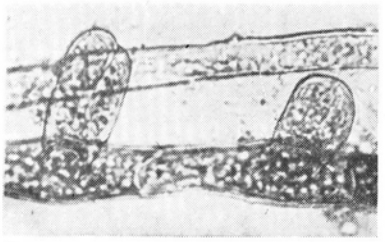




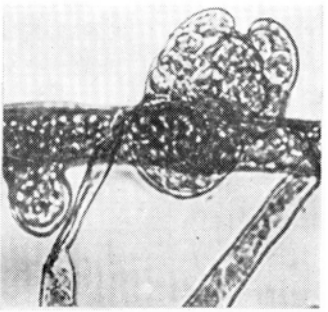


1. Leaf base regeneration: a) initial cell of basal part of leaf with growing protonema; b) basal part of leaf with 2 protonemata; c) basal part of leaf with 4 protonemata;
4. Branching protonemata: a) protonema in late developmental stage; b) chloronema — ch, c) caulonema — ca: d) caulonema with side branch

Plate III



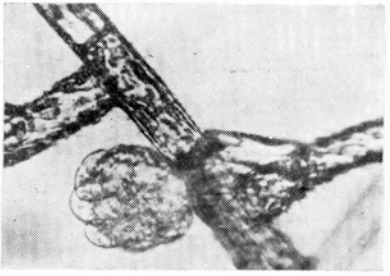
1a



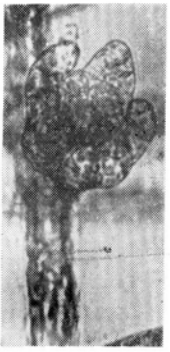
1b

1c

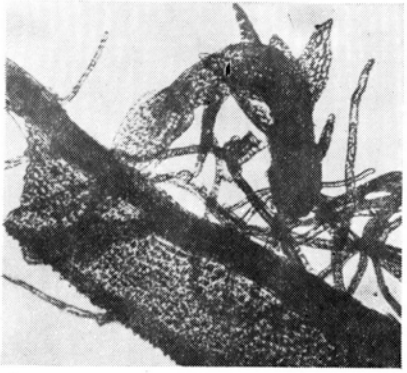
1d



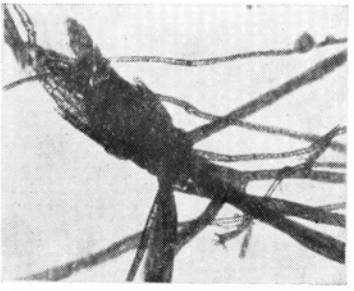
1e



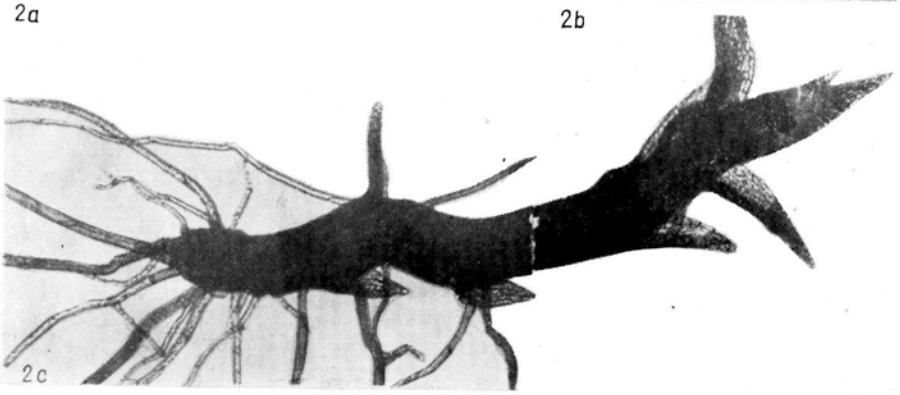
1f



2a



2b



2c

1. Gametophore development from protonema: a) first stage of bud formation; b, c) late stage of gametophore buds on protonema; d) leaf differentiation; e) leaf and stem differentiation. 2. Young gametophores formed from regenerating leaves: a) young gametophore conected with protonema and leaf; b) young gametophore on protonema; c) young gametophore with numerous protonemata, rhizoids — R, caulonema — c

Table 1

Experiment 1 and 2. Influence of 360 W and natural illumination as well of different substrates — agar, sand with Knop medium, peat and liquid 2% Knop medium on the percent of regenerating leaves from the basal and medial parts and from the rib

		Agar					Sand					Peat			H_2O
		Experiment 1				Exp. 2	Experiment 1				Exp. 2	Experiment 1		Exp. 2	Exp. 2
Substrate illumination		5 ml Knop/30 ml ag.		10 ml Knop/30 ml ag.		2% Knop/30 ml ag.	5 ml Knop/30 ml ag.		10 ml Knop/30 ml ag.		2% Knop/30 ml ag.	—		—	2% Knop/30 ml H_2O
		360 W × 10 g.	natural	360 W × 10 g.	natural	360 W × 10 g.	360 W × 10 g.	natural	360 W × 10 g.	natural	360 W × 10 g.	360 W × 10 g.	natural	360 W × 10 g.	360 W × 10 g.
Regeneration %	total	61.7	45.0	66.7	43.3	65.0	58.3	36.7	75.0	25.0	57.5	26.6	23.3	65.0	80.0
	rib	23.5	18.4	2.0	23.5	30.0	21.6	5.0	20.0	1.7	17.5	10.0	6.7	17.5	25.0
	basal	28.3	23.5	35.0	21.6	40.0	20.0	26.6	43.3	20.0	47.5	16.6	10.0	45.0	57.5
	medial	13.3	6.7	11.6	—	2.5	18.3	5.0	16.7	3.3	—	3.3	6.0	7.5	2.5

Table 2

Experiment 2. Per cent of leaves regenerating on agar with 2% Knop medium, on sand with 2% Knop medium, liquid 2% Knop medium and on peat at various observation times

Date of checking	Substrate			
	agar	sand	medium	peat
30.10.73	7.5	0	7.5	0
6.11.73	15.0	5.0	25.0	5.0
13.11.73	25.0	12.5	27.5	10.2
20.11.73	30.0	17.5	42.5	20.0
27.11.73	40.0	22.5	42.5	32.5
4.12.73	47.5	37.5	52.5	40.0
11.12.73	52.5	40.0	62.5	50.0
18.12.73	52.5	42.5	70.0	55.0
24.12.73	57.5	50.0	75.0	57.5
2.01.74	65.0	57.5	80.0	65.0

b) a caulonema, with slanting brown septa, numerous chloroplasts, a large amount of storage substances and a well visible nucleous (Plate II, 2c, 2d).

Numerous bulges appear on the protonema (Plate III, 1a, 1b), giving rise to new branchings or dividing to give a bud (Plate III 1b, 1c, 1d, 1e, 1f).

Owing to differentiation of the initial cell and its divisions "morula--shaped" stages from (Plate III, 1c, 1e) and then young plants.

The leaves of the young plants show morphological and anatomical differences as compared with those of mature plants; they have no ribs or verrucated cell walls.

From the basal parts of young plants developing owing to regeneration, protonemal filaments grow (Plate III, 2a, 2b, 2c) on which new plants arise.

CONCLUSIONS

Injured leaves of *Aulacomnium palustre* form differentiated protonemata (chlorenemata and caulonemata) on which gametophore buds develop and further foliated plants. On their tips "heads" appear with leaf-shaped gemmulae.

Regeneration is most intensive in the basal part of the leaf from 40.0% on agar with 2 per cent Knop's medium, to 57.5 per cent on 2 per cent liquid Knop's medium.

On the tested substrates a higher per cent of regenerating leaves was obtained under fluorescent tube illumination of 360 W for 10 h daily in the course of the entire experiment than under natural daylight.

Regeneration on peat and sand is delayed as compared with the same process on agar.

Vegetative reproduction and regeneration are the chief ways of multiplication which decide of the durability of populations of this species.

I wish to express my deep gratitude to prof. I. Rejment-Grochowska for her valuable adwice and help during the course of this work.

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Regeneracja mchów na przykładzie Aulacomnium palustre (Hedw.) Schwaegr

Streszczenie

Autorka prowadziła dwa doświadczenia na regenerację liści *Aulacomnium palustre*. Oderwane od łodyg liście hodowlane były na agarze i piasku z pożywką Knopa (Tabela 1), na torfie oraz na płynnej 2% pożywce Knopa. W doświadczeniu 1 zastosowano 2 warianty oświetlenia: jarzeniowe o mocy 360 W, światło naturalne; w doświadczeniu 2 na podłożu zasilanym 2% pożywką Knopa stosowano tylko światło jarzeniowe o mocy 360 W.

Z liści wyrastały dwa rodzaje protonemy — chloronema i kaulonema, których komórki dzieląc się tworzyły liczne rozgałęzienia i rozwijały się w młode gametofory.

Najbardziej aktywna w procesie regeneracji okazała się bazalna część liścia, w doświadczeniu 2 regenerowało z niej od 40% do 57,5% liści.

Światło o mocy 360 W przyspieszało regenerację. Na agarze w doświadczeniu 1 regenerowało ogółem 66,7% liści w oświetleniu jarzeniowym, a na takim samym podłożu przy oświetleniu naturalnym 43,3%; na piasku różnice te wynosiły 50%.