

On the development of gametophytes in *Funaria hygrometrica* and *Ceratodon purpureus* in liquid cultures

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In recent years many papers appeared concerning morphology and morphogenesis of moss gametophytes. Mosses are easy to cultivate and are characterized by striking differences in their developmental stages, i.e. protonema, gametophore and sporophyte stages. Much interest has been paid to the chemical factors determining transition from one stage to another, esp. the gametophore formation in the protonema. Several factors were found to be active in the formation of gametophore buds, e.g. light, sugar, auxins, gibberellic acid, kinetin and other derivatives of adenine (Bopp 1953, Gorton & Eakin 1957, Mitra & Allsopp 1959 A and B) and Patterson (1957) showed that the reactivity of bryophytes to auxin varies from species to species.

The present paper is a preliminary study of morphogenetic reactions to some growth factors of *Funaria hygrometrica* and *Ceratodon purpureus* in liquid cultures. The liquid cultures have the advantage of permitting an easy removal of the moss protonema from the medium and a ready washing free from it, which may be useful when a chemical analysis is to be made.

MATERIAL AND METHODS

Ripe capsules of *Funaria hygrometrica* Hedw. and *Ceratodon purpureus* (Hedw.) Brid. were collected in Stepnica at Zalew Szczeciński and on Ociemny Wierch in the Pieniny mountains resp., in summer 1960.

The capsules were first dipped in 95% ethanol for 1 min. and then in 0.2% HgCl_2 for 5 min. After washing with sterile water they were opened in sterile Petri dishes and small portions of spores transferred by an inoculation needle to culture tubes.

Kofler (1959) solution for *Funaria hygrometrica* was used as basal culture medium throughout the experiments, as it was also found to be the best for *Ceratodon purpureus* out of the three media tested

(Knop, Benecke and Kofler solutions). The 1% glucose, kinetin at 1 mg/l and indoleacetic acid (IAA) at 10 mg/l concentrations were employed in experimental media. The concentrations were chosen according to data of Mitra & Allsopp (1959 A and B) who found that 1% glucose and kinetin at 1 mg/l have a stimulating effect on bud formation and IAA at 10 mg/l completely inhibits budding in *Pohlia nutans*. The pH of the media was 5.6. The cultures were grown in light of two white fluorescent tubes at a temp. 20–25°C.

RESULTS

After six weeks of incubation, protonema with gametophores, if present, was filtered, washed and weighed after removing excess water with filter paper.

There was no germination in IAA solution at 10 mg/l.

The fresh weight was essentially the same in control cultures of both *Ceratodon* and *Funaria*. The 1% glucose caused a strong increase in fresh weight. The increase was stronger for *Ceratodon* when compared with that of *Funaria*. Kinetin at 1 mg/l was nearly without effect on the fresh weight of the mosses. The results are shown in Table 1.

Table 1
Fresh weight (mg/culture) of the mosses

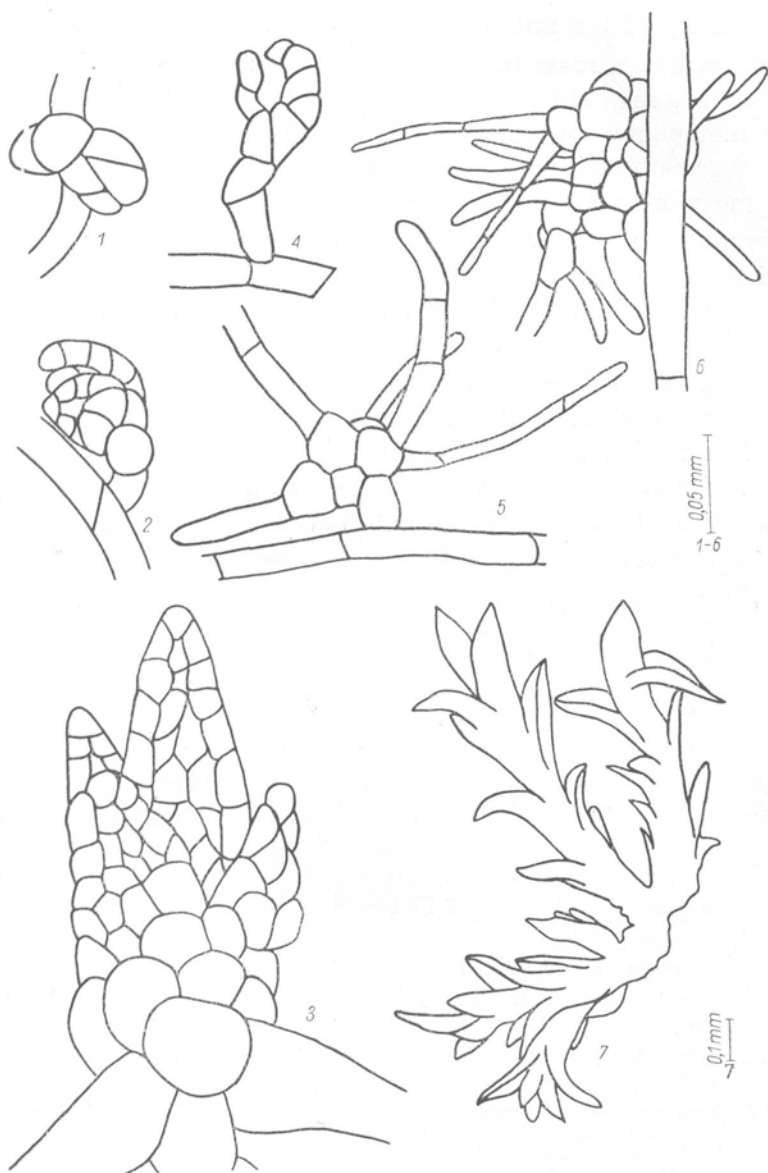
	<i>Ceratodon purpureus</i>	<i>Funaria hygrometrica</i>
Control	14.8	15.3
1% glucose	240.6	85.2
Kinetin 10^{-6}	15.2	12.6
IAA 10^{-5}	0.0	0.0

After four weeks the control cultures of *Funaria* showed an intensive bud and leafy shoot formation. No organized buds were seen in control cultures of *Ceratodon* at this time.

In *Funaria* the bud initial cell is very early and markedly formed and the leaves are early and well differentiated (figs. 1–3).

Ceratodon represents another type in respect to its morphogenesis in liquid cultures. After five weeks of culture, protonema consisted mainly of a typical chloronema, without caulonema or shoot buds. Some chloronema cells became spherical and in some cultures two-dimensional and three dimensional growths were observed, but without tendency to organized growth and with chloronema filaments growing out of unorganized cell masses (figs. 4–6). After three months, few buds and leafy

Plate I



Figs 1—3. *Funaria hygrometrica*: bud development in control cultures

Figs 4—6. *Ceratodon purpureus*: development of unorganized cell masses in young control cultures.

Fig. 7. *Ceratodon purpureus*: branched shoots in a medium containing kinetin

shoots appeared. The buds and the bases of leafy shoots resembled in some way moruloid masses of isodiametric cells. This indicated some difficulties of organized growth in early bud stages, presumably because of small activity of the initial cell (figs. 8—9).

Kinetin caused a mass induction of buds in both *Funaria* and *Ceratodon* in four weeks old cultures. In *Funaria*, however, further growth of the gametophores was inhibited. The buds had the tendency to a moruloid growth and later, after three months, new buds differentiated in these moruloid masses, resulting in branched forms (figs. 13—14).

In *Ceratodon*, moruloid character of young buds was still more indicated than in control cultures (fig. 10). After seven weeks, organized growth in buds and leafy shoots were seen. The moruloid bases of shoots and the shoots themselves were often branched (figs. 11—12).

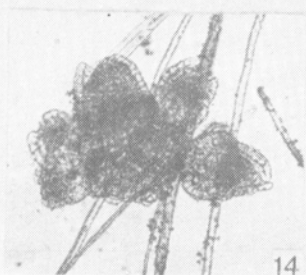
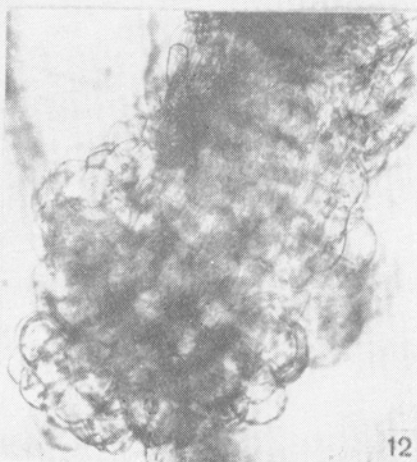
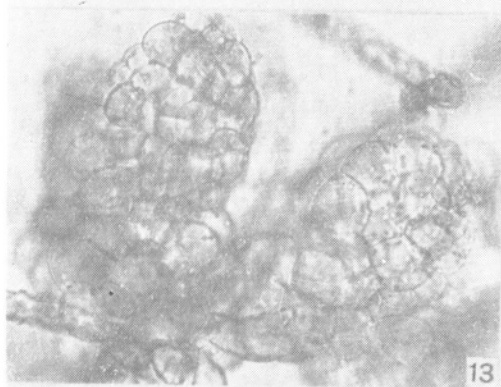
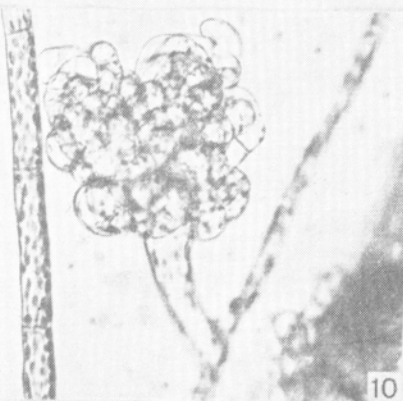
Glucose stimulated bud formation in *Funaria*, but was without effect in *Ceratodon* cultures. Protonema and gametophores of both *Funaria* and *Ceratodon* were brown coloured in glucose cultures. The leaves were markedly reduced in size (figs. 15—18). In young, four weeks old cultures, only few chloroplasts were observed in the cells and they were often degenerating. In older cultures the mosses were usually deprived of chlorophyll.

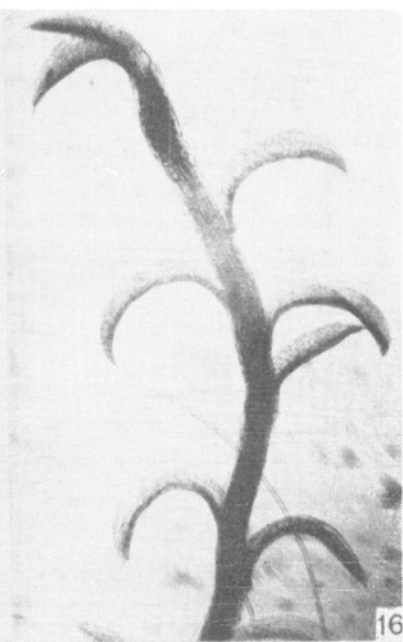
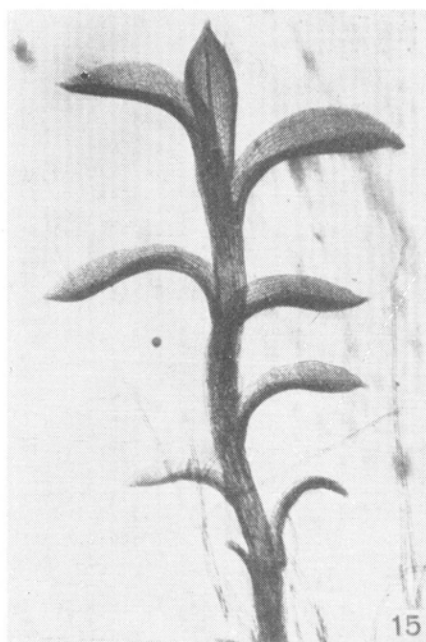
Plate II

- Figs 8—9. *Ceratodon purpureus*: a young bud (8) and a base of a grown shoot (9) in control medium (290 ×)
 Fig. 10. *Ceratodon purpureus*: a shoot bud in kinetin medium (290 ×)
 Figs 11—12. *Ceratodon purpureus*: leafy shoots (11) and a base of a shoot (12) grown in kinetin medium (fig. 11 — 58 ×, fig. 12 — 290 ×)
 Figs 13—14. *Funaria hygrometrica*: abnormal branched buds in kinetin medium, (fig. 13 — 290 ×, fig. 14 — 58 ×)

Plate III

- Figs 15—16. *Funaria hygrometrica*: shoots grown in control (15) and in glucose (16) media (20 ×)
 Figs 17—18. *Ceratodon purpureus*: shoots grown in control (17) and in glucose (18) media (20 ×)





DISCUSSION

Funaria hygrometrica and *Ceratodon purpureus* show marked differences in their development in sterile liquid cultures. *Funaria* forms an abundance of gametophores either on agar or in liquid media and in *Ceratodon* the gametophore formation is poor and slow. In *Funaria* the organizing activity of the apical cell results in early and well developed leafy buds and shoots. In *Ceratodon*, because of slow differentiation in its protonema, most of the stages in gametophore development described by Gorton and Eakin (1957) may be seen: filamental cells, spherical cells, two-dimensional and three-dimensional unorganized growths and organized leafy shoots. Regressions from two- and three-dimensional to one-dimensional filamentous type of growth were also observed.

Differences between two species were also conspicuous in their responses to glucose and kinetin. Bopp (1957) found that bud formation in *Funaria* was connected with the amount of protonema and suggested a possibility of an accumulation of a bud forming substance in the protonema. In our *Funaria*-experiments we also found more abundant bud formation in glucose cultures with increased fresh weight. In *Ceratodon*, however, glucose caused a great increase in fresh weight without affecting bud formation.

Kinetin is a known factor stimulating bud formation in mosses (Gorton & Eakin 1957, Mitra & Allsopp 1959). Supplying the cultures of *Ceratodon* with kinetin, abundant bud formation may be achieved. This resulted at first in some "moruloid" character of buds, but after some time normally appearing leafy shoots were formed which eventually branched later on. In *Funaria* cultures, bud formation was also greatly stimulated by kinetin, but their further development was inhibited, they became moruloid in appearance, and did not develop into leafy shoots.

Mitra & Allsopp (1959 B) found that *Pohlia nutans* may grow on media supplied with IAA at 10 mg/l, although bud formation is completely inhibited. Hurel-Py (1948, 1953) found that protonema of *Funaria* may grow on media containing NAA (naphtaleneacetic acid) at 10 mg/l. In our experiments there were no signs of moss development of either *Funaria* or *Ceratodon* in media containing IAA at 10 mg/l. The 1% glucose had some stimulatory effect on bud formation in *Funaria* but not in *Ceratodon*. The leaves of shoots formed were much reduced in this concentration, thus indicating that *Funaria* and *Ceratodon* are more sensitive to sugar than *Pohlia* which was found by Mitra & Allsopp (1959 A) to develop optimally in 1% conc. of glucose.

As a result of our preliminary experiments *Ceratodon* is found to be of a special interest for further investigations of moss development. Its

protonema hardly produces leafy shoots in normal culture conditions, but formation of buds may be achieved by some special treatment as, e.g., by kinetin supply.

SUMMARY

The development of the gametophytes in *Funaria hygrometrica* and *Ceratodon purpureus* in various liquid media was investigated.

Funaria produced protonema and gametophores in mineral Kofler medium. The 1% glucose stimulated both fresh weight and the number of gametophores formed but it reduced the size of leaves and chlorophyll content in them. Kinetin at 1 mg/l increased greatly the number of buds formed but they were of rather abnormal "moruloid" appearance and did not develop further into leafy shoots.

Ceratodon produced protonema in mineral medium, but gametophores developed only occasionally in this medium. Fresh weight of the protonema was much increased in 1% glucose medium but gametophore production was not affected. Abundant formation of buds and leafy shoots was obtained in medium containing kinetin at 1 mg/l.

Morphogenetic differences are thus shown between *Funaria* and *Ceratodon*, the latter being of special interest for further investigations.

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