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The effect of kinetin on gametophyte development and protein synthesis in the moss *Ceratodon purpureus*

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Recent investigations have shown a very strong morphogenetic activity of kinetin in the development of gametophyte in mosses. A. "bud-forming" activity of kinetin causes a mass initiation of gametophore buds in the protonema and in the moss *Ceratodon purpureus* kinetin has been shown to induce bud formation even in the dark being thus able to replace such morphogenetically active factor as light (Szweykowska 1964).

There are important data on the relantionship between kinetin and protein synthesis (Maciejewska-Potapczykowa and Keller 1958; Mothes, Engelbrecht and Kulajewa 1959; Thimann and Laloraya 1960). It is also generally accepted that morphogenetic processes, cell and tissue differetiations are connected with qualitative as well as quantitative changes in protein metabolism. Hotta and Osawa (1957) investigated changes in protein concentration in the cells during the development of a young fern gametophyte and found that during its primary one-dimensional, linear protonema-like growth the protein concentration slightly decreased whereas the tranistion to the two-dimensional, plate-like prothalium growth was accompanied by a rapid increase in protein concentration.

In the development off moss gametophyte, the one-dimensional growth pattern is represented in the filamentous protonema and the formation of gametophores ("leafy shoots") is characterized by a change in the direction of cell division leading to a three-dimensional growth pattern.

The purpose of this study was to investigate the influence of kinetin on protein formation in the moss gametophyte and to learn if changes in protein concentration similar to those observed in fern gametophyte can be demonstrated in the development of mosses.

MATERIAL AND METHODS

C. purpureus (Hedw.) Bird. plants with spore capsules were collected in Góry Bialskie mts. (Sudetes, Poland) in spring 1961. Sterile moss cultures were grown from spores. Ripe capsules were dipped in 95 per cent ethanol for 1 min. and then in 0.2 per cent $HgCl_2$ for 5 minutes,

washed with water, opened under sterile conditions and small portions of spores transferred to 100 ml Erlenmayer flasks containing 30 ml of nutrient medium. As the nutrient medium mineral Kofler (1959) solution of the following composition was used:

H_2O	ad 1000 ml	KH_2PO_4	0.20 g
$Ca(NO_3)_2 \cdot 4H_2O$	0.25 g	$Na_2HPO_4 \cdot 2H_2O$	0.05 g
K_2SO_4	0.25 g	$FeCl_3 \cdot 6H_2O$	0.001 g
$MgSO_4 \cdot 7H_2O$	0.25 g		

Micro-nutrients were supplied in the form of Heller's solution:

$FeCl_3 \cdot 6H_2O$	1.00 mg	$CuSO_4 \cdot 5H_2O$	0.03 mg
$ZnSO_4 \cdot 7H_2O$	1.00 mg	$AlCl_3$	0.03 mg
H_3BO_3	1.00 mg	$NiCl_2 \cdot 6H_2O$	0.03 mg
$MnSO_4 \cdot 4H_2O$	0.10 mg	KJ	0.01 mg

Glucose at 0.25 per cent and kinetin at 1 mg/l were added to some experimental media. In some experiments, kinetin was added to the culture flasks after a definite time of incubation. The cultures were grown in light of white fluorescent tubes and at a room temperature about 18°C.

Every determination was made on 3—5 parallel cultures. The cultured plant material was filtered off the nutrient solution, dried with filter paper and fresh weight estimated. For dry weight determination, the fresh material was dried at 105°C. to a constant weight.

The protein fraction was seperated according to Hotta and Osawa (1957). The plant material, kept in 80 per cent ethanol, was centrifuged, homogenized and left for 15 hours in 10 per cent trichloroacetic acid (TCA) at 2°C. The precipitate was centrifuged, suspended in 5 per cent TCA and heated for 20 min. at 90°C to remove nucleic acids. After centrifuging and washing with 5 per cent TCA, the precipitate was used as protein fraction for estimating protein nitrogen by a micro-Kjeldahl method according to Humphries (1956). The precipitates were transferred to test tubes and digested in an aluminium block after the addition of H_2SO_4 (0.5 ml for 500 mg fresh weight) and a catalyst mixture ($CuSO_4 + K_2SO_4 + SeO_2$ in a ratio 1:8:1). The samples were first boiled at 150°C till water evaporated and later at about 320°C untill the digest cleared. The digest was distilled in a Parnas Wagner apparatus and the ammonia trapped into 5 ml of 2 per cent boric acid. It was titrated with n/28 HCl in the presence of an indicator mixture (methyl red at 0.1 per cent and methylene blue at 0.05 per cent (till the green colour changed to violet). One ml of HCl corresponded to 0.5 mg of protein nitrogen.

RESULTS

C. purpureus is an autotrophic plant capable of developing in an inorganic medium. Its growth, however, is distinctly increased by the addition of glucose, in light as well as in the dark, the optimal concentration of glucose being 1 per cent (Fig. 1). In this concentration, however,

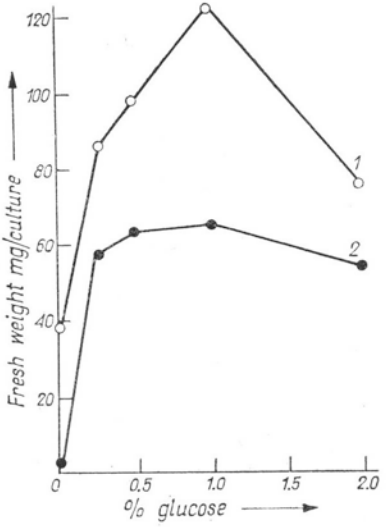

Fig. 1. The effect of glucose on growth of *C. purpureus* cultures
1 — light; *2* — dark

the cultures became soon brown coloured, gametophores were of generally abnormal appearence and their leaves reduced. In further experiments a suboptimal concentration 0.25 per cent was used in which growth exceeded more tahn 100 per cent that in inorganic control yet culture colour and gametophore development were not affected.

Kinetin exerts an inhibiting effect on growth of the cultures in an early phase of their development (Figs. 2 and 3). In later stages, the effect of kinetin on growth depended on whether the cultures were grown on inorganic or organic media. In inorganic media, there was no difference in fresh weight between control cultures and those supplied with kinetin (Fig. 2). In media supplied with glucose a distinct growth promoting activity of kinetin was observed (Fig. 3). In inorganic media obviously the insufficiency of organic materials was limiting factor for growth. The growth promoting activity of kinetin in organic media was to some extent due to an increased water uptake by the cells which was shown by dry weight determinations: in controls, average dry weight amounted to about 20 per cent of the fresh weight and in kinetin to 15 per cent only. In our previous study (Szweykowska and Maćkowiak 1962), we observed that moss buds formed and grown in kinetin had a tendency to transform in moruloid masses consisting of

spherical, swollen cells. The effect of kinetin on cell enlargement is also reported by Miller (1961) and Maciejewska-Potapczykowa and Keller (1958) found a stimulating effect of kinetin on water accumulation in cells. However, the growth promoting activity of kinetin consisted not merely in increased water uptake by cells of *C. pur-*

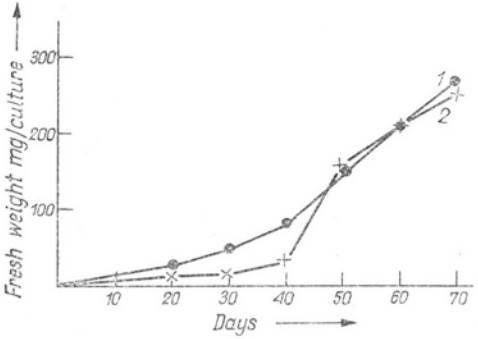

Fig. 2. Growth of cultures on inorganic medium
1 — control; *2* — kinetin

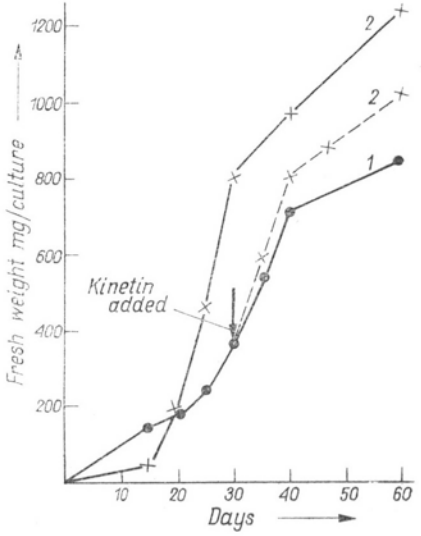

Fig. 3. Growth of cultures on organic medium (0.25 per cent glucose)
1 — control; *2* — kinetin

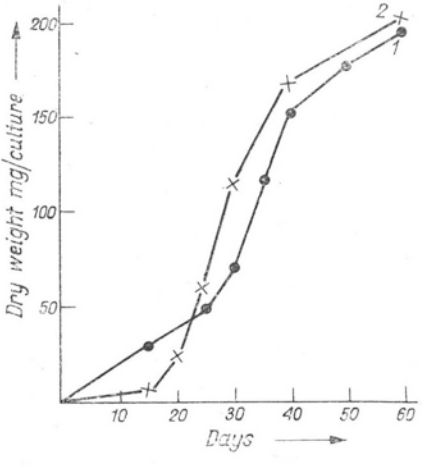

Fig. 4. Dry weight content of cultures (organic medium)
1 — control; *2* — kinetin

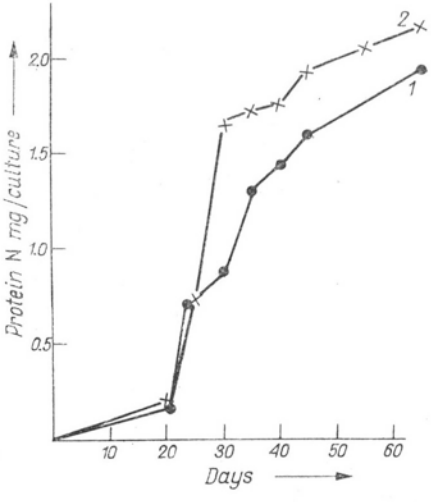

Fig. 5. Protein synthesis in cultures (organic medium)
1 — control; *2* — kinetin

pureus culture. The absolute values of dry weight per culture were higher in kinetin than in control media (Fig. 4) and also the protein content was higher in cultures supplied with kinetin (Fig. 5).

The growth promoting activity of kinetin is especially distinct in dark cultures. In these cultures grown without access of light growth is

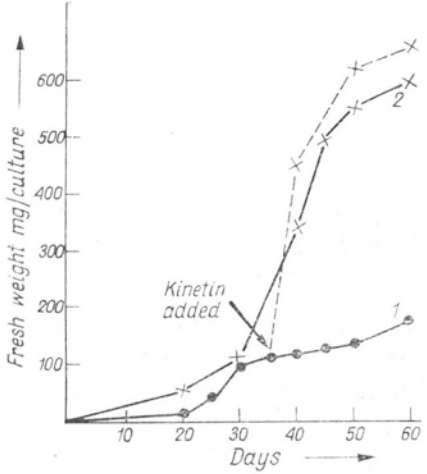

Fig. 6. Growth of cultures in the dark (organic medium)
1 — control; *2* — kinetin

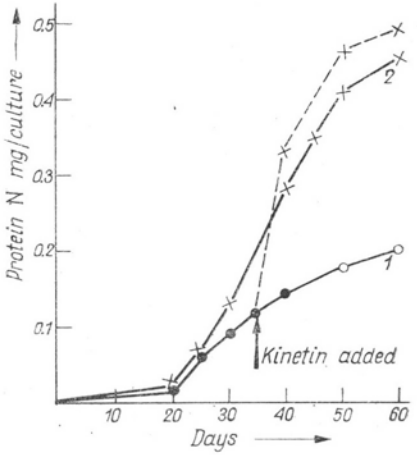

Fig. 7. Protein synthesis in cultures grown in the dark (organic medium)
1 — control; *2* — kinetin

3—4 times better in kinetin media as indicated by higher fresh and dry weights and higher protein content of cultures (Fig. 6 and 7).

The promoting effect of kinetin on bud differentation in mosses has been found in several investigations (Gorton and Eakin 1957; Mitra and Allsopp 1959; Bopp 1962; Szweykowska 1962, 1963). In the present study, buds appeared in control cultures after about 50 days on inorganic media, after 30 days in organic media and in dark cultures single buds were observed after 50 days. In media supplied with kinetin, buds appeared in great number in first days of culture sinultaneously with protonema development, in dark cultures somewhat later, after 25 days. Kinetin added to a control medium in which protonema grew already for some longer time induced in few days mass differentiation of buds. Thus the time of bud appereance in the protonema is much shortened and the number of buds much enlarged by kinetin.

The protein determinations showed that no rapid change of protein concentration in dry weight accompanied gametophore initiation in *Ceratodon* culture. The protein concentration expressed in mgs per dry weight increased in the first phase of culture development and later steadily

decreased with no marked increase during bud formation (Fig. 8). When related to the fresh weight, the protein concentration was high in the first phase of protonema development, then gradually lowered, increased

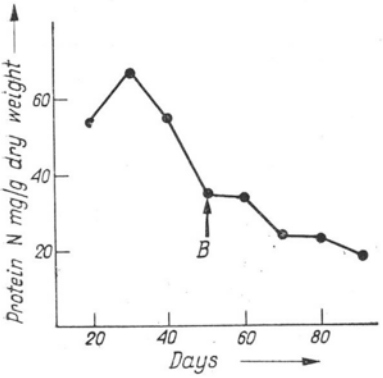

Fig. 8. Protein content per dry weight during culture development (inorganic medium)
B — buds

again during bud initiation and once more decreased while gametophores were growing and differentiating (Figs. 9 and 10). In the presence of kinetin, there was a distinct decrease in protein concentration as shown in figs. 9 and 10. Also an addition of kinetin to a developing control culture soon resulted in a strong decrease of protein concentration together with mass bud initiation (Fig. 10). These results, and especially the effect of kinetin, suggest that the changes in protein concentration are rather not connected with the direction of cell division in one- or

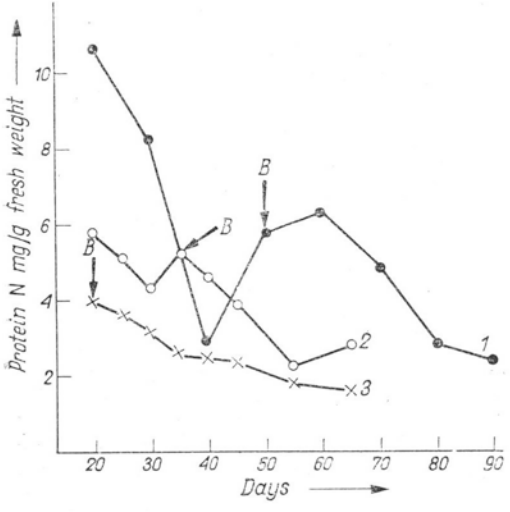

Fig. 9. Protein content per fresh weight during the development of cultures
1 — inorganic; *2* — organic; *3* — organic + kinetin; *B* — buds

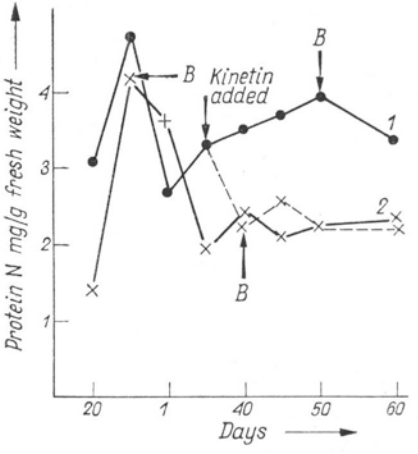

Fig. 10. Protein content per fresh weight during the development of cultures in the dark (organic medium)
1 — control; *2* — kinetin; *B* — buds

three-dimensional growth patern of protonema or gametophores but depend more on meristematic activity of the cultures. This may be especially demonstrated in the experiment with dark cultures which developed relatively slowly. The protein concentration of the culture at first increased in the phase of intensive protonema growth, then a rapid decrease followed aging of the protonema. During gametophore formation accompanied by initiation of new meristematic centres with active cell divisions, the protein concentration of the culture gradually increased an then fell again during gametophore differentiation and aging. Kinetin induced rapid cell division and initiation of many buds together with an enhanced water uptake, cell differentiation and aging-because the further development of buds was to some extent inhibited. This is believed to be the reason that the protein concentration in kinetin cultures was relatively low (Fig. 9.) and an addition of kinetin to a developing culture resulted in a rapid decrease of it (Fig. 10).

DISCUSSION

Hotta and Osawa (1957) estimated protein content in the course of gametophyte development in the fern *Dryopteris erythrospora* and found that the primary one-dimestional linear growth, whether normal or artificially induced, was characterized by decrease of protein concentration whereas the two-dimestional, plate-like growth was accompanied by its rapid increase. They suggested that the change in the direction of cell divisions resulting in a two-dimensional growth pattern of a prothallium, is causally connected with the rapid synthesis of protein.

The present investigation carried out with *C. purpureus* has shown that during the gametophyte development the protein concentration is highest in the first phase of protonema growth, increases also during bud initiation and is decreasing during protonema and gametophore differentiation. These changes, however, are believed to be connected rather with changing meristematic activity followed by aging of the culture than with different orientation of cell division plates in protonema and forming gametophores.

The bud initiation in the protonema is strongly stimulated by kinetin. Kinetin markedly enhances this process as well as induces formation of an enormous number of buds when compared with the control. The growth and protein synthesis are also much stimulated by kinetin, the protein content of kinetin cultures is higher than that of controls. It is interesting, however, that the decrease in protein concentration during the development is much stronger in kinetin than in control cultures. In the development of *Ceratodon*, kinetin would thus first of all play a role of a factor of cell division, cell enlargement and three-dimensional

growth. The intensity of cell divisions and cell enlargements are higher than promotion of protein synthesis, the dry weight and protein concentration being thus much lower in kinetin than in control cultures.

SUMMARY

The effect of kinetin on growth, differentiation and protein synthesis during the development of the gametophyte in a moss *C. purpureus* was investigated. Kinetin promotes growth (estimated as fresh and dry weight of the culture) and protein synthesis and stimulates strongly gametophore bud initiation. It is a very active factor of cell division and three-dimensional growth and of cell enlargement by a water uptake. It strongly enhances the culture differentiation, causing a more rapid decrease of protein concentration in the culture when compared with that of control.

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Wpływ kinetyny na rozwój gametofitu i syntezę białka u mchu Ceratodon purpureus

Streszczenie

Zbadano wpływ kinetyny na wzrost, różnicowanie i syntezę białka w trakcie rozwoju gametofitu u mchu *C. purpureus*. Kinetyna pobudza wzrost (oznaczany jako świeża i sucha masa kultury) i syntezę białka oraz stymuluje silnie zakładanie się pączków gametoforów. Jest bardzo aktywnym czynnikiem podziałów komórkowych i wzrostu trójwymiarowego (podziały w różnych płaszczyznach podziałowych) oraz powiększania się komórek dzięki pobieraniu wody. Przyspiesza również silnie różnicowanie się kultury i powoduje, iż koncentracja białka (zawartość na jednostkę świeżej masy) spada szybciej niż na kontroli.