

The effect of various cytokinins and other factors on the protonemal cell divisions and the induction of gametophores in *Ceratodon purpureus*

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Abstract

The cytokinins specifically induce formation of gametophore buds in the protonema of *Ceratodon purpureus*. The response in this species is less sensitive than in *Funaria hygrometrica*, but is independent of light. The cytokinins also stimulate protonemal cell divisions, this response, however, is not specific and affected by many other factors.

INTRODUCTION

The formation of a large number of gametophore buds in the protonema of *Funaria hygrometrica* has been found to be a specific response to the addition of cytokinins to the medium (Hahn and Bopp 1968; Szweykowska, Schneider and Prusińska 1969). The cytokinin-induced stimulation of cell divisions in cultures of isolated protonema cells of the same moss species proved to be non-specific (Szweykowska et al. 1971), indicating that in the bud induction more than a simple stimulation of cell divisions is involved.

The purpose of this work was to investigate the effect of cytokinins on bud induction and protonemal cell divisions in *Ceratodon purpureus*, with respect to the sensitivity and specificity of these responses. *Ceratodon purpureus* has a much lower ability to spontaneous bud formation than *Funaria hygrometrica* in laboratory cultures which may be of advantage in using this moss species in cytokinin bioassays as well as in various experiments with cytokinins. Therefore, a better knowledge of this plant material with respect to its response to cytokinins seemed to be necessary.

MATERIAL AND METHODS

The spore capsules of the moss *Ceratodon purpureus* (L. ap. Hedw.) Brid. were collected in the Botanical Garden in Poznań in 1968. Sterile culture of the protonema was prepared by a procedure described earlier (Szweykowska and Handszu

1965). A clone culture was obtained by transferring small fragment of a protonema filament on fresh agar medium. The culture was then propagated vegetatively by transplantations of the protonema to new culture media in Erlenmayer flasks. The medium consisted of a mineral solution solidified with agar (0.8%). The cultures grew under continuous white fluorescent light of about 1000 lux and at a temperature of about 25°C. Even after a prolonged time of growth, the stock cultures remained at the protonemal stage of development, with only very occasional formation of gametophores.

A. The bud-induction response. The protonema was pre-cultivated for 2 weeks on agar medium enriched with glucose at 0.25%. Samples of the protonema were then transferred to short, wide tubes containing 10 ml of nutrient solution (mineral salts and glucose at 0.1%). After 7 days, 1 ml portions of the test substance solutions were aseptically added to the tubes. Four culture tubes were used for each experimental variant. One set of cultures was kept under light, and a parallel set in a complete darkness. After 5 days, the cultures were examined under a dissection microscope.

B. The cell-division response. The culture of protonema cells was carried out on small cylinders of nutrient agar. This method has been found more useful than the previously applied culture in hanging-drops (Szweykowska et al. 1971). Cells divided more vigorously giving after 3 days of culture a better yield than

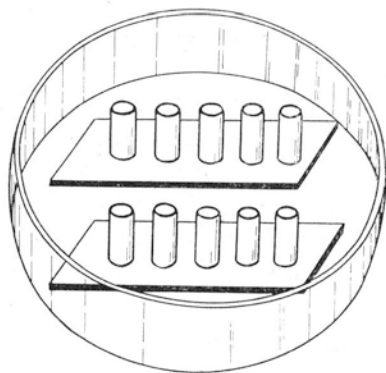


Fig. 1. The culture of cells on cylinders of nutrient agar

after 7 days in hanging-drops. Cylinders 5 mm in diameter and 10 mm high were cut out from the nutrient agar by means of a cork-borer. Cellophane discs of the same diameter were placed on the top of each cylinder. Five agar cylinders were mounted on a glass slide and two such slides placed in a Petri dish padded with wet filter paper (Fig. 1). Ten agar cylinders were prepared for each experimental variant. Prior to cell inoculations, open dishes were sterilized under a mercury lamp. The protonema was precultivated for 10 days on cellophane discs (Bopp, Jahn und Klein 1964) placed on the surface of 20 ml of agar medium in 100 ml Erlenmayer flasks. From the protonema filaments placed in a drop of nutrient solution,

single apical cells were excised under the microscope and each transferred on the top of an agar cylinder. The cells were cultivated in the culture room in the same conditions of temperature and light as the stock cultures. After 3 days of growth, the cells produced filaments of various length which were examined under the microscope. The cell number produced during this time was taken as a measure of the cell division rate.

RESULTS

The effect of cytokinins on bud formation

Following adenine derivatives known for their high biological activity were tested: N⁶-furfuryladenine (kinetin, KIN), N⁶-benzyladenine (6-BA), N⁶-(Δ²-isopentenyl)adenine (6-IPA), and 6-phenylureidopurine (6-FUP), (Fig. 2). They were all active as bud inducers, their activity increasing in the order:



The lowest concentrations which induced a large number of buds were as follows:

6-IPA	—	0.5 μM
6-BA	—	1.0 μM
KIN	—	5.0 μM
6-FUP	—	100.0 μM

The relative activities of various cytokinins were similar as in the case of bud-induction in *Funaria hygrometrica*, although the concentrations necessary for bud-induction in *Ceratodon* were ten times higher as compared with *Funaria* (Szweykowska, Schneider and Prusińska 1969).

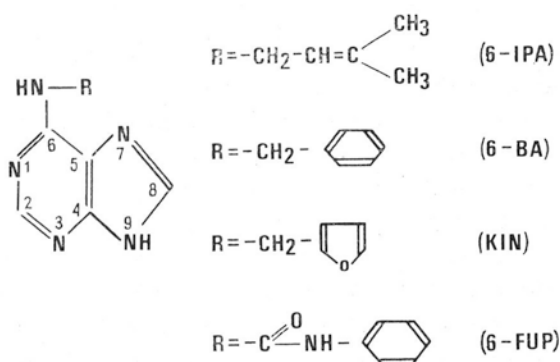


Fig. 2. The chemical structure of 4 cytokinins used in experiments

Formation of gametophore buds under the influence of cytokinins occurred both in the light and in complete darkness. In this respect *Ceratodon* differs from *Funaria* which is unable to form buds in the absence of light. High concentrations of cytokinins do not inhibit the bud formation. In a special experiment, kinetin

was added at several concentrations between 10–10 000 μM . None of the concentrations used inhibited gametophore formation, although at 1000 and 10 000 μM degeneration symptoms occurred in both protonema and bud cells.

From among the other than N^6 -monosubstituted adenines, 1-isopentenyladenine (1-IPA), 9-isopentenyladenine (9-IPA), and 1-methyl- N^6 -benzyladenine (1-M-6-BA) were tested. Similarly as in *Funaria*, 9-IPA and 1-M-6-BA were ineffective in inducing gametophore formation in concentrations between 1–100 μM . The 1-IPA was active in a relatively high concentration of 10 μM , however, this result cannot be conclusive as the 1-substituted adenines are known for their easy rearrangement to N^6 -substituted derivatives (Leonard et al. 1966). The 1-IPA and 9-IPA at 10 μM when added to the medium together with 6-IPA at 0.5 μM seemed to increase the effect of 6-IPA alone — the buds were more numerous and larger.

Other purine and pyrimidine derivatives like adenine, guanine, cytosine, uracil, adenosine and deoxyadenosine were inactive as bud inducers when tested in concs. up to 100 μM .

Gibberellic acid (GA) in concs. between 0.1–10 μM produced no response in the protonema. Unlike in *Funaria*, GA did not show any synergistic effect with kinetin. In the conditions of the bud-induction test used in this study, there was also no gametophore formation in the presence of 3-indolylacetic acid (IAA) at 0.01–1 μM . IAA, however, showed a synergistic effect with cytokinins, similarly as it was observed previously for *Funaria* (Szweykowska, Schneider and Prusińska 1969). In the presence of IAA at 0.5 μM , a strong bud induction was observed in kinetin concentration of 1 μM which alone did not suffice to produce buds.

The effect of cytokinins on protonemal cell divisions

Various cytokinins added to the agar medium stimulated cell divisions of the protonema cells showing the same order of activity as in the bud-induction response (Fig. 3). Also the concentrations of various cytokinins in which cell divisions reached their maximum were the same as those necessary for the appearance of the bud-response. However, the similarity between the two responses extends no further. Several substances, inactive as bud-inducers, stimulated cell divisions, some of them to a much higher degree than cytokinins. A distinct stimulation appeared with 1-M-6-BA at 10 μM (Fig. 4), with IAA at 1 μM , and with GA at 2 μM (Fig. 5). A very high rate of cell divisions appeared in cultures to which adenine or adenosine at 100 μM were added (Fig. 6). Also in the presence of glucose, protonema cells divided very vigorously (Fig. 7).

On the other hand, the adenine derivatives monosubstituted in a position other than N^6 proved to be highly inhibitory to cell divisions. Isopentenyl adenine derivatives monosubstituted in positions 1-, 3-, or 9-, resp. (1-IPA, 3-IPA, 9-IPA) and benzyl derivatives substituted in positions 1-, and 3-, resp (1-BA, 3-BA) depressed cell divisions beginning with a relatively low, 0.001 μM concentration (Fig. 8).

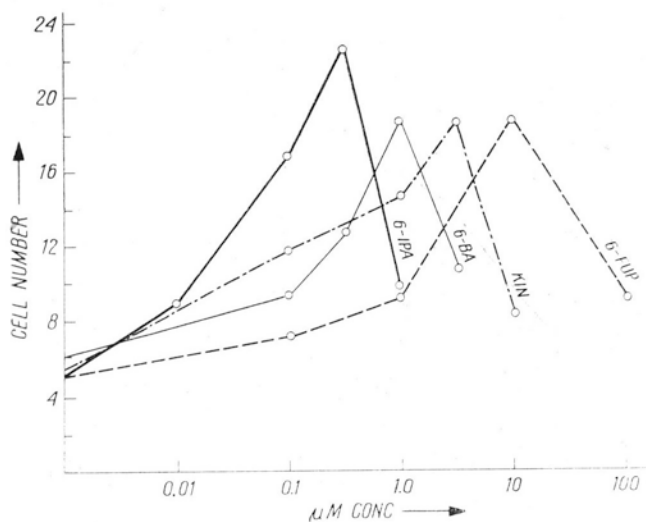


Fig. 3

The effect of various cytokinins on cell divisions

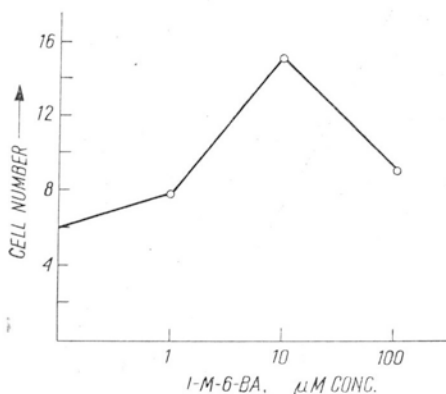


Fig. 4. The effect of 1-M-6-BA on cell divisions

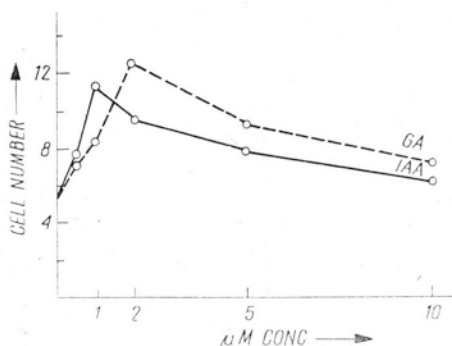


Fig. 5. The effect of IAA and GA on cell divisions

As indicated above in this paper, these substances, at concs. up to 10 μM , showed no harm effects when used together with cytokinins in the bud induction test, and 1-IPA acted even as bud inducer when applied alone at 10 μM . It is thus evident that the responses of cells dividing to produce a protonema filament are very different from those giving rise to gametophore buds.

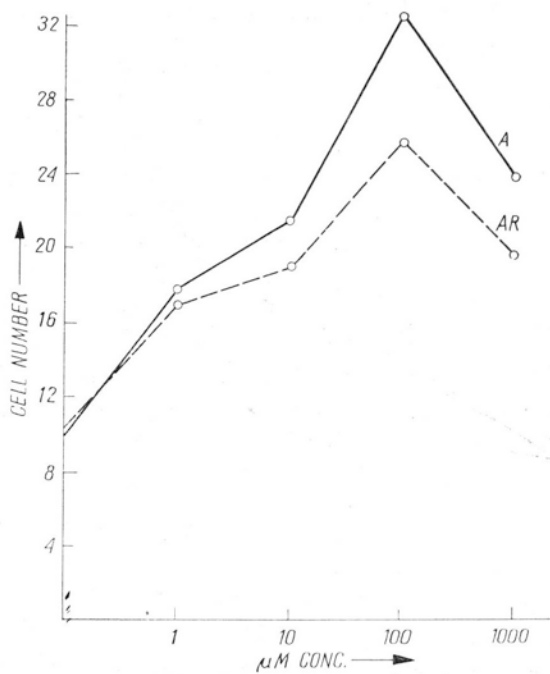


Fig. 6. The effect of adenine (A) and adenosine (AR) on cell divisions

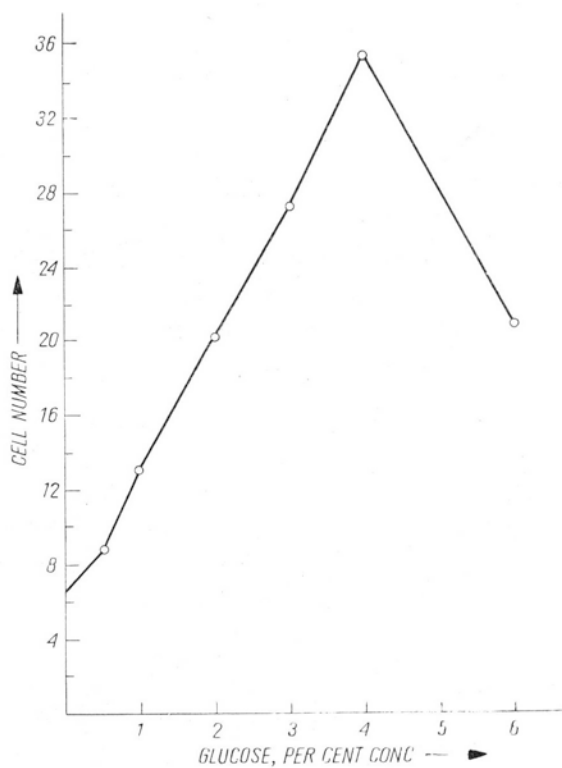


Fig. 7. The effect of glucose on cell divisions

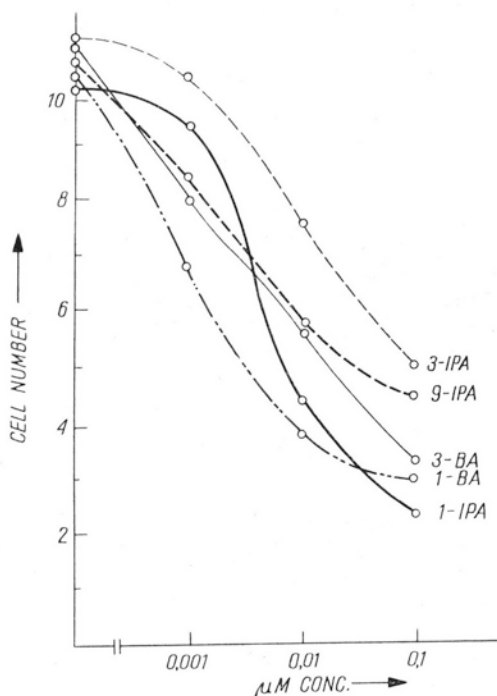


Fig. 8. The effect of various cytokinin-like adenine derivatives monosubstituted in other than N⁶ position on divisions of cells

DISCUSSION

The specificity of the bud induction response to cytokinins is in *Ceratodon* similar as in *Funaria*. However, the sensitivity of the response is in *Ceratodon* about 10 times lower than in *Funaria*. This may be caused by a lower level of endogenous cytokinins in this species as shown by its much weaker tendency to spontaneous gametophore formation. In conditions of laboratory cultures, *Funaria* forms gametophores about 20 days after the inoculation of spores in a cytokinin-free medium, whereas *Ceratodon* may be cultivated in laboratory conditions for months as a protonema without forming gametophores. This feature of *Ceratodon*, however, may be of advantage in longer-lasting experiments in which a rigorously gametophore-free control is required. It also facilitates the use of a genetically uniform material for various experiments, in form of a protonemal stock culture constituting a vegetatively propagated clone.

The response of isolated protonema cells to cytokinins is in *Ceratodon* also similar to that in *Funaria*: cells divide more vigorously, showing a similar sensitivity to cytokinins as in the bud-induction test, but the response is not specific in the conditions used. It seems to be a basically quantitative growth response depending on several and various factors. The bud-induction instead seems to be principally

a qualitative, morphogenetic response, only indirectly related to the stimulation of growth.

The inhibition of protonemal cell divisions by cytokinin-analogues of the type of adenine derivatives monosubstituted at other than N⁶ position needs further investigation.

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SUMMARY

In the protonema of *Ceratodon purpureus*, cytokinins are specific factors inducing formation of a large number of gametophore buds, similarly as it has been previously found for *Funaria hygrometrica*. The response in *Ceratodon* is less sensitive than in *Funaria*, requiring 10 times higher concentrations of cytokinins, but is independent of light. An important advantage of *Ceratodon purpureus* as a model organism in cytokinin investigations is its lower ability to spontaneous gametophore formation in conditions of laboratory culture. It can be cultivated for months in form of a vegetatively propagated protonema which permits to have a genetically uniform clone material for various experiments.

The cytokinins also stimulate protonemal cell divisions, this response, however, is not specifically related to cytokinins and shows several inconsistencies with the bud induction response. It is concluded that in *Ceratodon*, similarly as in *Funaria*, the cell division rate is a basically quantitative growth response, affected by various, unspecific factors, contrary to the bud-induction which constitutes a qualitative morphogenetic response to factors more specific than those required for growth.

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Wpływ różnych cytokinin i innych czynników na protonemalne podziały komórkowe i na indukcję gametoforów u Ceratodon purpureus

Streszczenie

Cytokiny są specyficznymi czynnikami indukującymi masowe pojawienie się pączków gametoforowych w splątku *Ceratodon purpureus*, podobnie jak to wykazano poprzednio dla *Funaria hygrometrica*. Reakcja ta jest u *Ceratodona* mniej czuła niż u *Funarii*, wymaga bowiem 10-krotnie wyższych stężeń cytokinin, ale niezależna jest od światła. Ważną zaletą gatunku *Ceratodon purpureus* jako organizmu modelowego dla badania cytokinin jest jego mniejsza zdolność do samorzutnego tworzenia gametoforów w warunkach kultury laboratoryjnej. Może on być hodowany przez wiele miesięcy w postaci wegetatywnie przeszczepianego splątka, co pozwala na posiadanie jednolitego genetycznie materiału dla różnych doświadczeń.

Cytokiny stymulują również protonemalne podziały komórkowe, ta reakcja jednak nie jest specyficznie związana z cytokininami i wykazuje szereg niezgodności z reakcją pączkotwórczą. Z rezultatów pracy wynika, że u *Ceratodon*, podobnie jak u *Funaria*, intensywność podziałów komórkowych w splątku jest reakcją wzrostową zależną od różnych niespecyficznych czynników, w przeciwieństwie do indukcji pączków gametoforowych, która stanowi jakościową, morfogenetyczną reakcję na czynniki bardziej specyficzne aniżeli te, które wymagane są dla wzrostu.

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