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Studies on the specificity and sensitivity of the bud-induction response to cytokinins in the protonema of *Funaria hygrometrica*

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The induction of gametophore buds in the protonema of mosses is one of the most striking biological effects of cytokinins. Bauer (1966) used this effect in estimating kinetin-like activity of a substance isolated from a moss callus, and Bopp and Diekmann (1967) found a quantitative relationship between kinetin concentration and bud formation in isolated caulonema filaments. In the present study, the specificity and sensitivity of this cytokinin effect was investigated in order to test its usefulness as a basis for a relatively simple cytokinin bioassay.

MATERIAL AND METHODS

Protonema cultures of *Funaria hygrometrica* grown from spores in liquid inorganic medium of Kofler (1959) were used in the experiments. The protonema was cultured in 100 ml Erlenmayer flasks containing 20 ml of nutrient solution, in continuous white fluorescent light of about 1000 lux. After 10—12 days of growth, the

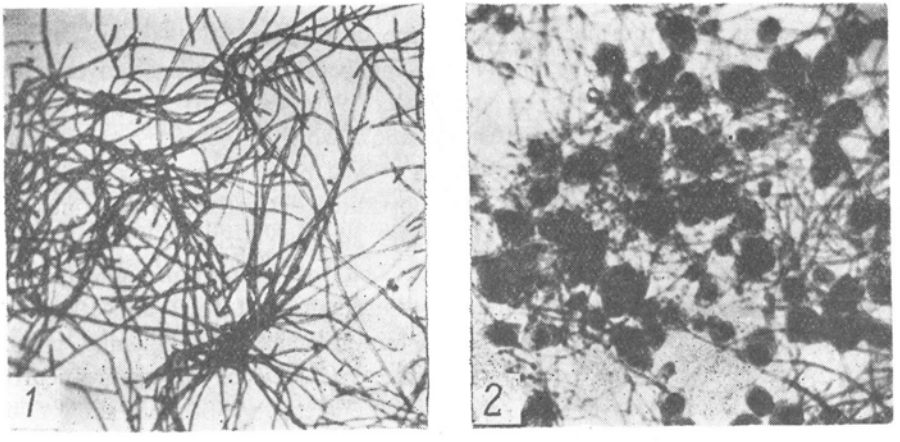


Fig. 1—2 The bud--inducing effect of cytokinins
1 — cytokinin-free solution; *2* — kinetin in 0.5 μM/l concentration
Efekt paczkotwórczy cytokinin
1 — roztwór bez cytokininy; *2* — kinetyna w stężeniu 0.5 μM/l

protonema was filtered and protonema samples were transferred to wide test tubes containing 5 ml of medium into which the tested substances were included. After 5—7 days, the bud inducing activity of the test solutions was checked with a dissection microscope. In controls, none or only single buds were present at that time, whereas a mass appearence of buds was characteristic for solutions with cytokinin activity (fig. 1—2).

RESULTS

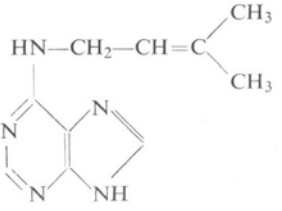
1. The effect of various cytokinins

Three cytokinins: kinetin, 6-benzyladenine (BA) and 6-isopentenyladenine (IPA), in concentrations from 0.001 to 1 μM/l were tested and compared for their bud inducing effect. All three were active as bud inducers, IPA beginning with 0.01, BA with 0.05 and kinetin with 0.1 μM/l concentrations. The order of their activity (see table no. 1) was similar to that found with tobacco bioassay (Skoog et al. 1967).

Table 1

The bud induction response to various cytokinins and the synergistic effect of GA and IAA

μM/l	0.005	0.01	0.05	0.1
IPA	—	+	+	+
BA	—	—	+	+
Kin.	—	—	—	+
Kin.+ GA or IAA	—	+	+	+



6-Isopentenyladenine

2. The effect of other purine and pyrimidine derivatives

With the exception of some phenylurea and benzimidazole derivatives (Bruce and Zwar 1966; Kefford, Bruce and Zwar 1966; Engelbrecht, Bräuniger and Koine 1967; Kuraishi and Yamaki 1967) which in some bioassays possess some cytokinin activity, the high activity, as proved by the tobacco callus bioassay (Skoog et al. 1967), is restricted to N-6 substituted derivatives of 6-aminopurine. The purine compounds, and particularly the adenine derivatives, are therefore of special importance with respect to the specificity of a cytokinin bioassay. Following purine and pyrimidine derivatives, in concs. of 1, 10, 100 and 1000 μM/l, were tested for their possible cytokinin-like, bud-inducing effect: adenine, guanine, cytosine, uracil, adenosine, deoxyadenosine, 9-(2,3-dideoxy-D-erythro-pentosyl) adenine and 9-(2,3-dideoxy-D-threo-pentosyl) adenine. None of them showed bud-inducing effect characteristic for cytokinins.

3. The effect of other growth regulators

A positive effect of auxins and gibberellins on bud formation has been reported for some mosses (Mitra and Allsopp 1959, Szweykowska 1962). In *Funaria*

hygrometrica, Bopp (1953) found a slight stimulation of bud formation by 3-indolylacetic acid (IAA), and a similar effect of gibberellic acid (GA) has been found by Jahn (1964), and in some culture conditions also by Prusińska, Schneider and Szweykowska (1968). It was thus necessary to examine the action of auxins and gibberellins as possible bud inducers or substances interferring in the bud inducing effect of cytokinins. In one set of experiments, the effects of IAA (0.01, 0.05, 0.1, 1 and 10 μM/l), of 2,4-dichlorophenoxyacetic acid (2,4-D, 0.01, 0.1, 1 and 10 μM/l), and of GA (0.1, 1, 10 and 100 μM/l) on bud formation in 10 and 12 days old protonemas were tested. None of the substances used showed any bud-inducing effect during 7 days. The highest concentrations were toxic and caused disintegration of the protonema cells. In another set of experiments, IAA and GA in the same concentrations as before were tested in combinations with kinetin in 0.01, 0.1 and 0.5 μM/l. Basal control contained no active substances and kinetin controls contained kinetin alone. Large number of buds appeared in media containing kinetin in 0.1 and 0.5 μM/l, no buds being present in basal control and kinetin control 0.01 μM/l. In the presence of IAA in 0.05 or 0.1 μM/l, or of GA in 0.1 or 1 μM/l, the bud inducing effect of kinetin appeared in as low concentration of the latter as 0.01 μM/l. This showed that although IAA and GA are no bud inducers by themselves, they act synergistically and increase the sensitivity of the protonema to the cytokinins. With kinetin alone, the bud induction occurred beginning with 0.1 μM/l, in the presence of IAA or GA it appeared already in 0.01 μM/l of kinetin, i.e. the sensitivity of the protonema to kinetin became tenfold increased (table no. 1).

CONCLUSIONS

Protonema of *Funaria hygrometrica* pre-cultured for 10—12 days on a liquid, cytokinin-free medium and transferred to media containing cytokinins produces large number of buds in 3—7 days. The relative activities of various cytokinins are similar to those found in the tobacco bioassay. Several pyrimidine and purine derivatives other than cytokinins are inactive. Auxins and gibberellic acid in cytokinin-free medium are also inactive, although in the presence of cytokinins they show a synergistic effect. The bud-induction response of mosses to cytokinins seems thus to be quite specific. The sensitivity of the response is also relatively high: using a medium containing additionally GA (1μM/l) or IAA (0.1 μM/l), the effect is produced by 0.01 μM/l of kinetin, and few mililiters of solution are needed to obtain the effect.

The bud induction response of the protonema of *Funaria hygrometrica* seems thus to present a good basis for a cytokinin bioassay. The described procedure is particularly suitable for qualitative estimations, but may also be used for approximate quantitative determinations by preparing a series of concentrations and finding the lowest one producing the bud induction.

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Badania nad specyfiką i czułością reakcji pączkotwórczej splątka mchu Funaria hygrometrica na cytokininy

STRESZCZENIE

Splątek *Funarii* hodowany przez 10—12 dni na płynnej kontrolnej pożywce, a następnie przeniesiony na pożywki zawierające cytokininy tworzy masowo pączki gametoforowe w ciągu 3—7 dni. Względna aktywność różnych cytokinin (kinetyna, 6-benzyloadenina, 6-izopentenyloadenina) jest tu podobna jak w teście tytoniowym. Szereg pochodnych pirymidynowych oraz nie-cytokininowych pochodnych purynowych wykazał zupełny brak aktywności pączkotwórczej. Nieaktywne są także auksyny oraz kwas giberelowy w nieobecności cytokinin, chociaż wykazują efekt synergistyczny, zwiększając czułość reakcji splątka na cytokininy. Tak więc reakcja pączkotwórcza mchów na cytokininy wykazuje dużą specyficzność. Czułość reakcji jest także stosunkowo wysoka: stosując pożywkę zawierającą GA (1 μM/l) lub IAA (0.1 μM/l) efekt pączkotwórczy występuje już przy stężeniu kinetyny 0.01 μM/l pożywki, przy czym dla otrzymania efektu wystarcza kilka mililitrów pożywki.

Reakcja pączkotwórcza splątka *Funarii* wydaje się stanowić dobrą podstawę dla stosunkowo prostego i szybkiego testu na cytokininy. Opisana w pracy procedura nadaje się szczególnie do oznaczeń jakościowych, jednakże stosując szereg stężeń badanych roztworów i znajdując najniższe wywołujące efekt pączkotwórczy można także użyć jej do przybliżonych oznaczeń ilościowych.