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Short Communication

Comparative Activity of Isomers of Zeatin and Ribosyl-Zeatin on *Funaria hygrometrica*[1]

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ABSTRACT

The activities of isomers of zeatin, ribosyl-zeatin, and 6-(γ,γ-dimethylallylamino)purine (i^{6}Ade) on the moss *Funaria hygrometrica* are compared by measuring the ability of the cytokinins to induce callus or gametophores. The *cis*- and *trans*-ribosyl-zeatins were inactive, and therefore this kind of bioassay cannot be used as evidence for the presence or absence of a cytokinin in tests on natural products.

A bioassay for cytokinins using *Funaria hygrometrica* as developed by Hahn and Bopp (1) has been used as a means of detecting cytokinin-active substances. This test is based on the finding that buds on the protonemata of this moss are induced by cytokinins. The test is highly sensitive and gives an "all or none" response if no cytokinin is present in the medium. As shown by Szweykowska *et al.* (5), it generally gives the same relative order of activity for different cytokinins as does the tobacco bioassay. It has been assumed that all cytokinin substances are active in this test.

This assay was used by Wood *et al.* (7, 8) to differentiate between cytokinins and a cell division factor they called cytokinesin and which they had extracted from crown-gall tumor cells of *Vinca rosea*. This cell division-promoting factor, as described by Wood (6), had been found to be comparatively inactive in inducing buds on the *Funaria* test when compared with the cytokinin benzyladenine. Adding the cytokinesin to benzyladenine did not increase the activity of the cytokinin (7). Hence the conclusion was made that a new class of cell division-regulating substances whose synthesis may be stimulated by cytokinins and which are directly involved in promoting cell division had been isolated in a form that was not contaminated by a cytokinin (6).

Recently, Miller (2) showed that a cell division-promoting material isolated from tumor tissue of *Vinca rosea* and from the culture medium in which it was growing was ribosyl-*trans*-zeatin and that this nucleoside was the major cell division factor present. This conclusion is in contradiction to the study of Wood *et al.* (7, 8) who used the results of the *Funaria* test to make the distinction between known cytokinins and cytokinesins and for designating cytokinesin as a separate class of growth-regulating substances. It was therefore of great interest to determine what the relative activities were of isomers of zeatin and ribosyl-zeatin in the *Funaria* test.

MATERIALS AND METHODS

A sporangium of *Funaria hygrometrica* was sterilized in 70% alcohol and opened aseptically onto sterile filter paper. The spores were transferred to a flask containing 150 ml of modified Nebel and Naylor medium as described by Spiess *et al.* (4). After 14 days, 1 ml of the culture was transferred to 50 × 12 mm Petri dishes. Cytokinin and additional medium were added. Cultures were maintained in a growth chamber at 25 C in a 16/8 hr light/dark cycle. The first appearance of structures recognized as buds was determined by periodic examination under a dissecting microscope. Seventeen days after cytokinins had been added to the culture, counts of callus or gametophores were made. Three dishes with approximately 15 plants each were examined for each treatment.

Sporangia of *Funaria* and zeatin compounds which had been synthesized by Dr. Nelson J. Leonard (University of Illinois, Urbana) were kindly supplied by Dr. Folke Skoog (University of Wisconsin). The cytokinin 6-(γ,γ-dimethylallylamino)purine (i^{6}Ade) was obtained from Calbiochem.

RESULTS

Funaria hygrometrica does not respond to all cytokinins by producing buds and subsequent callus or gametophores. From Table I it is clear that in three replicate experiments ribosyl-*trans*-zeatin at 10^{-6} M was almost ineffective in producing buds and later gametophores when compared to the controls. *trans*-Zeatin (10^{-6} M) was very active in producing callus masses which remained as callus with large swollen cells. The fact that a concentration effect was not involved is shown by the experiment in which *trans*-zeatin at 10^{-8} M was more active than ribosyl-*trans*-zeatin at 10^{-6} M. The callus masses produced by the lower concentration of *trans*-zeatin organized and formed some clusters of normal gametophores. *cis*-Zeatin and ribosyl-*cis*-zeatin at both concentrations were less active than *trans*-zeatin. Any structures that were produced became single normal gametophores (Fig. 1). The cytokinin i^{6}Ade was 1.6 to 2.4 times more active than *trans*-zeatin at 10^{-6} M and similarly more active at the lower concentration in this test. The structures produced with the higher concentration of i^{6}Ade remained as large callus masses with enlarged cells. As with *trans*-zeatin at 10^{-8} M, some organization to produce leafy gametophores in clusters resulted with the lower concentration.

Not only were the number and the morphology of the structures different with the cytokinins used but also the time

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at which initials of these structures first appeared varied as shown in Table II. Again *trans*-zeatin and i^{6}Ade were the most active, producing recognizable initials at 11 to 12 days after cytokinin had been added. At 10^{-8} M, these two cytokinins had produced buds after 12 to 14 days. For plants treated with other cytokinins, no bud initials appeared until after 16 days with one exception. At this time bud initials also appeared in those control cultures which did produce gametophores.

DISCUSSION

It was established in this study that ribosyl-*trans*-zeatin, ribosyl-*cis*-zeatin, and *cis*-zeatin are almost inactive in inducing buds on *Funaria*. The few buds induced by these three substances became normal gametophores. The cytokinin i^{6}Ade was 1.6 to 2.4 times as effective in producing callus as *trans*-zeatin, which in turn was significantly effective. Not only did the quantity of structures vary but also the time to first appearance and the ultimate morphology of the structures varied with the cytokinins used.

The *Funaria* test used in these experiments is a modification of that developed by Hahn and Bopp (1). Complete plants were maintained in a liquid medium, and the appearance and the ultimate fate of the buds were noted. The *Funaria* test used by Wood *et al.* (7) also differed from the one Hahn and

Table I. *Number of Structures Produced per Plant 17 Days after Addition of Cytokinins*

Treatment	Replicates		
	1	2	3
M			
Control	0.20	0.75	0.00
trans-Zeatin			
10^{-6}	5.32	2.85	2.40
10^{-8}	1.30	0.50	0.80
Ribosyl-*trans*-zeatin			
10^{-6}	0.37	0.90	0.00
10^{-8}	0.13	0.54	0.00
cis-Zeatin			
10^{-6}	0.66	1.37	0.11
10^{-8}	0.05	0.42	0.00
Ribosyl-*cis*-zeatin			
10^{-6}	0.50	0.47	0.36
10^{-8}	0.01	0.40	0.04
i^{6}Ade			
10^{-6}	8.75	5.80	5.90
10^{-8}	2.38	1.22	1.00

Table II. *Number of Days to First Appearance of Bud Initials after Addition of Cytokinins*

Treatment	Replicates		
	1	2	3
M			
Control	16	16	
trans-Zeatin			
10^{-6}	12	12	11
10^{-8}	12	12	14
Ribosyl-*trans*-zeatin			
10^{-6}	16	16	
10^{-8}	16	16	
cis-Zeatin			
10^{-6}	16	12	16
10^{-8}	16	16	
Ribosyl-*cis*-zeatin			
10^{-6}	16	16	16
10^{-8}	16	16	16
i^{6}Ade			
10^{-6}	12	12	11
10^{-8}	12	12	14

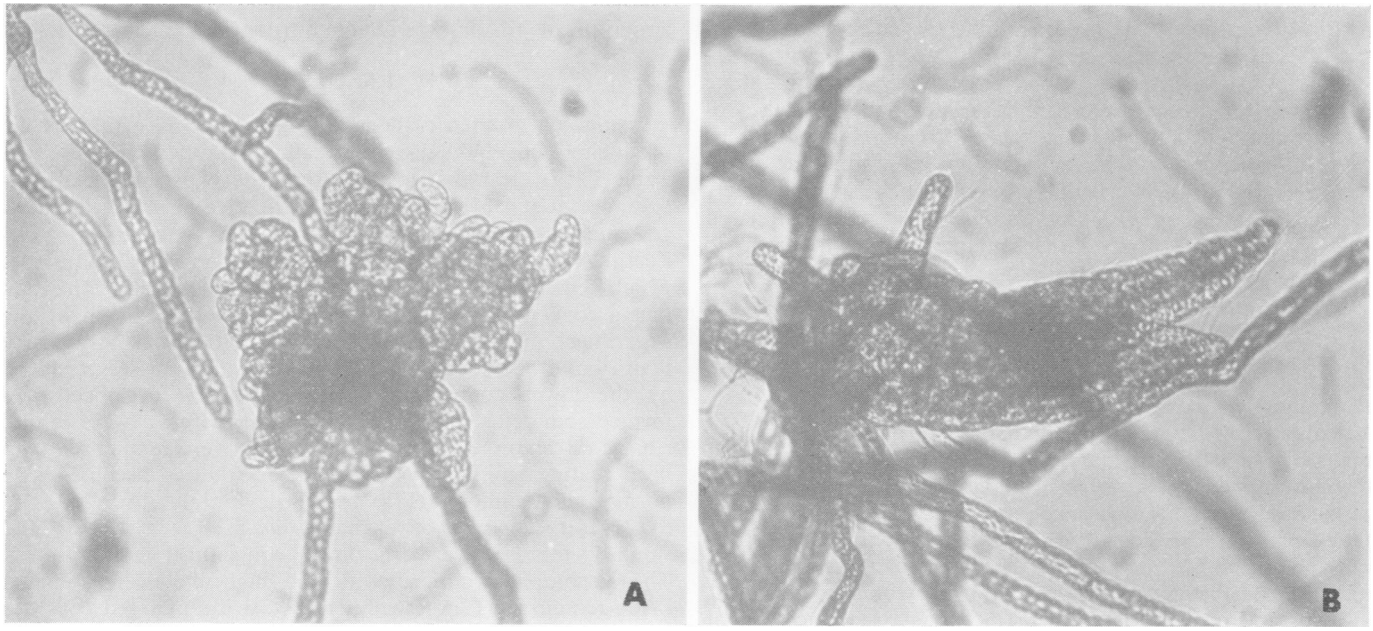

FIG. 1. Developmental structures resulting from treatment with cytokinins. A: Large callus with swollen cells as result of treatment with *trans*-zeatin or i^{6}Ade 10^{-6} molar; B: young normal gametophore as found in controls and plants treated with *cis*-zeatin or ribosyl-zeatin isomers × 60.

Bopp used, in that the former used complete protonemata and the latter used 10 cell sections from the protonemata. The magnitude of response varied 200-fold between the two experiments. The fact that the conditions of the test were not responsible for the low or lack of activity of ribosyl-zeatins and *cis*-zeatin is confirmed by Dr. Hans Kende (Michigan State University) who used a more conventional *Funaria* test and also found ribosyl-zeatin to be inactive (personal communication).

It is of interest to note that in tests of geometric and position isomers of zeatin activity using the tobacco callus bioassay, Schmitz *et al.* (3) found that *trans*-zeatin was the most effective.

The evidence reported here indicates that the *Funaria* test is not a valid indicator of the presence or absence of cytokinins as Wood *et al.* (7) have proposed. Ribosyl-*trans*-zeatin, which was not detected by this assay, has been shown to be the major cytokinin produced by *Vinca rosea* crown-gall tissues (2). The claims reported by Wood *et al.* (6–8) that a cell division-promoting factor, 3,7-alkyl-2-alkylthio-6-purinone, is present as the major substance regulating cell division in this tissue cannot be made on the basis of the *Funaria* test. Ribosyl-*trans*-zeatin may be the cell division-regulating substance in this tissue. The *Funaria* test may be specific for cytokinins, but the lack of response does not prove the absence of a cytokinin in a test solution.

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