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Differential Cytokinin Structure-Activity Relationships in *Phaseolus*[1]

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ABSTRACT

The activities of eight cytokinins in promoting callus growth were tested in two *Phaseolus* genotypes, *P. vulgaris* L. var. Great Northern, and *P. lunatus* L. var. Kingston. The structural feature which contributes to the major genotypic difference in cytokinin structure-activity relationships is the presence or absence of a double bond at the 2,3-position of the isoprenoid N^6 side chain. In Kingston, *trans*-zeatin was 3-fold more active than dihydrozeatin and 30-fold more active than *cis*-zeatin. The activities of N^6-(Δ^2-isopentenyl)adenine and N^6-isopentyladenine were nearly the same. In Great Northern, however, dihydrozeatin was at least 30-fold more active than both *trans*-zeatin and *cis*-zeatin, and N^6-isopentyladenine was 100-fold more active than N^6-(Δ^2-isopentenyl)adenine. The results suggest the possibility of employing cytokinin structure-activity relationships in distinguishing genotypic differences in cytokinin function and metabolism.

The structure-activity relationships of cytokinins have been investigated in detail by Skoog and Leonard and their associates using the tobacco callus bioassay system (2, 9, 10). The data for other cytokinin bioassay systems are much less extensive, but it is clear that the relative activities of cytokinins may vary in different bioassays, depending on both the type of biological response examined and the particular plant material selected for study. The causes of such differences in structure-activity relationships are not certain, although differences in cytokinin uptake, metabolism, and possible differences in the structural requirements for cytokinin activity at the site(s) of action in different plant materials may be involved.

In an attempt to identify physiological traits of potential value in investigations of the genetic regulation of cytokinin metabolism and function, we are examining cytokinin structure-activity relationships in cytokinin-dependent tissue culture lines derived from a number of *Phaseolus* genotypes. We report here the results of tests of the activities of eight cytokinins in promoting the growth of callus tissue derived from *P. vulgaris* var. Great Northern and *P. lunatus* var. Kingston.

MATERIALS AND METHODS

Chemicals. Zeatin,[2] N^6-(Δ^2-isopentenyl)adenine, N^6-hexyladenine, N^6-benzyladenine, and kinetin were obtained from Sigma; *cis*-zeatin and dihydrozeatin were from Calbiochem. N^6-Isopentyladenine was kindly provided by F. Skoog. Picloram was a gift from Dow Chemical.

Plant Materials. The genotypes *P. vulgaris* L. var. Great Northern and *P. lunatus* L. var. Kingston were used as experimental materials. Tissue cultures were established from the hypocotyl tissue of 5-day-old seedlings as described previously (5).

Tissue Culture Medium. Medium for *Phaseolus* callus tissues consisted of mineral nutrients as described by Murashige and Skoog (6) with the following organic substances added: sucrose (30 g/l), *myo*-inositol (100 mg/l), thiamine·HCl (1 mg/l), nicotinic acid (5 mg/l), pyridoxine·HCl (0.5 mg/l), and picloram (2.5 μM). Kinetin (5 μM) was included in the medium used for stock cultures. The pH of the medium was adjusted to 5.7 and Difco Bacto-agar (10 g/l) was added. The medium was dispensed into 125-ml Erlenmeyer flasks (50 ml/flask) and autoclaved at 120 C for 15 min. The choice of picloram as an auxin source was based on the broad range of concentrations of this compound that are effective in promoting callus growth of *Phaseolus* tissues (5).

Structure-Activity Tests. For structure-activity tests, appropriate amounts of cytokinins were dissolved in dimethylsulfoxide (7) and added to tissue culture flasks containing autoclaved basal medium (0.025 ml of dimethylsulfoxide solution/flask) prior to solidification of the medium. Four replicate flasks were used for each cytokinin concentration tested. Three pieces of callus weighing close to 25 mg each were planted per flask. Tissues were harvested and weighed after 28 days of growth at 28 C in the dark. The effects of the cytokinins on callus growth were determined in the second passage of the callus. The tests were repeated using newly established cultures. An exception to this was the testing of N^6-isopentyladenine, which was not repeated due to the limited quantity of the chemical available.

RESULTS

The structures of the cytokinins tested are shown in Figure 1. The activities of these cytokinins in promoting callus growth of *P. vulgaris* var. Great Northern and *P. lunatus* var. Kingston are compared in Figures 2, 3, and 4.

The activities of *trans*-zeatin, dihydrozeatin, and *cis*-zeatin in the two genotypes are shown in Figure 2. In Kingston (Fig. 2B), the relative activities of the three cytokinins were similar

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[2] Abbreviations: zeatin, *trans*-zeatin; *t*-(ioh[4]) ^{6}Ade: 6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)purine; *cis*-zeatin, *c*-(ioh[4])^{6}Ade: 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)purine; dihydrozeatin, (ipnoh[4])^{6}Ade: 6-(4-hydroxy-3-methylbutylamino(purine; i^6Ade: N^6-(Δ^2-isopentenyl)-adenine, 6-(3-methyl-2-butenylamino)purine; ipn^{6}Ade: N^6-isopentyladenine, 6-(3-methylbutylamino)purine; hx^{6}Ade: N^6-hexyladenine, 6-hexylaminopurine; bzl^{6}Ade: N^6-benzyladenine, 6-benzylaminopurine; kinetin, fr^{6}Ade: N^6-furfuryladenine, 6-furfurylaminopurine; picloram: 4-amino-3,5,6-trichloropicolinic acid.

to their activities in the tobacco callus bioassay system (3, 4, 8). *Trans*-zeatin was the most active of the three compounds. Dihydrozeatin was approximately 3-fold less active than *trans*-zeatin, and *cis*-zeatin was about 30-fold less active than its *trans* isomer. However, in Great Northern (Fig. 2A), the activity of dihydrozeatin was at least 30-fold greater than that of both *trans*- and *cis*-zeatin, with the two isomers exhibiting identical and relatively weak activity. It should be noted that the primary difference between the two tissues was in their response to *trans*-zeatin, although dihydrozeatin was slightly more active in Great Northern than in Kingston.

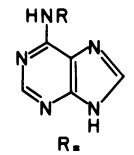
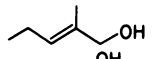
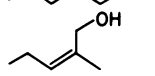
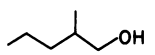
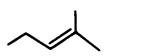
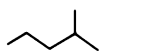
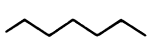
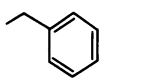
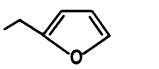
CYTOKININ	STRUCTURE
Name abbreviation	
Zeatin t-(ioh⁴)⁶Ade	
cis-Zeatin c-(ioh⁴)⁶Ade	
Dihydrozeatin (ipnoh⁴)⁶Ade	
N⁶-(Δ²-Isopentenyl)adenine i⁶Ade	
N⁶-Isopentyladenine ipn⁶Ade	
N⁶-Hexyladenine hx⁶Ade	
N⁶-Benzyladenine bzl⁶Ade	
Kinetin fr⁶Ade	

FIG. 1. Chemical structures of cytokinins tested.

To test further the effect of a double bond in the N^6 side chain on cytokinin activity as measured with the two types of *Phaseolus* callus tissue, the activity of N^6-(Δ^2-isopentenyl)adenine was compared with the activity of N^6-isopentyladenine (Fig. 3). The activity of N^6-isopentyladenine was 100-fold greater than that of N^6-(Δ^2-isopentenyl)adenine in promoting the growth of Great Northern tissue (Fig. 3A). N^6-Hexyladenine, another cytokinin bearing a saturated side chain, was also more active than N^6-(Δ^2-isopentenyl)adenine in this system. In Kingston (Fig. 3B), N^6-(Δ^2-isopentenyl)adenine and N^6-isopentyladenine were approximately equally active and were 10-fold more active than N^6-hexyladenine.

The effects of N^6-benzyladenine on callus growth of the two genotypes are shown in Figure 4. The growth response of N^6-(Δ^2-isopentenyl)adenine is included for comparison. N^6-Benzyladenine was more active than kinetin in both genotypes. In Great Northern (Fig. 4A), N^6-benzyladenine was the most active of all cytokinins tested. In Kingston (Fig. 4B), N^6-benzyladenine had approximately the same activity as N^6-(Δ^2-isopentenyl)adenine and was less active than *trans*-zeatin and dihydrozeatin. In this variety, N^6-hexyladenine, kinetin, and *cis*-zeatin were the least active of all cytokinins tested.

A slight increase in fresh wt (1.6 g/flask) was observed in the control tissues of Great Northern. There was no growth of Kingston tissue on medium devoid of cytokinin.

DISCUSSION

The most interesting difference in cytokinin activities between the two genotypes is seen with cytokinins bearing isoprenoid side chains. In *P. lunatus* var. Kingston, *trans*-zeatin and N^6-(Δ^2-isopentenyl)adenine are either more active than the cytokinins bearing the corresponding saturated side chains (dihydrozeatin and N^6-isopentyladenine) or at least as active. These results are similar to those observed in the tobacco bioassay

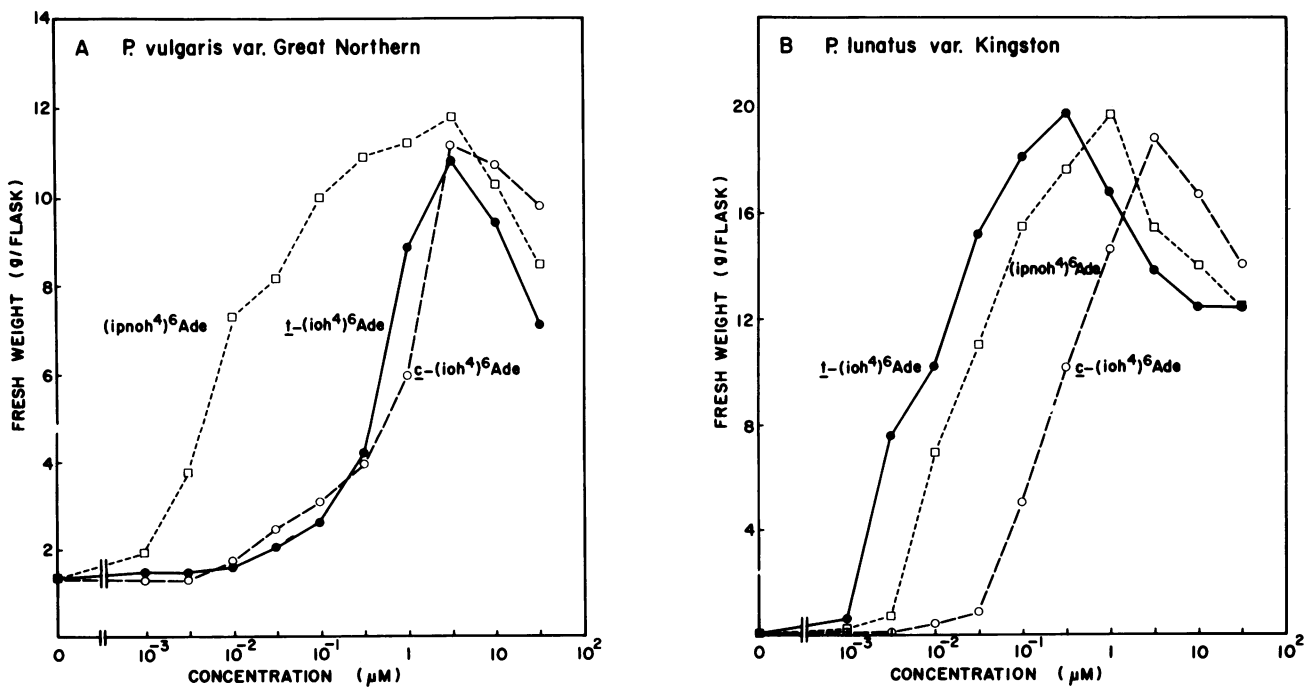


FIG. 2. Cytokinin activities of *trans*-zeatin [*t*-(ioh⁴)⁶Ade], *cis*-zeatin [*c*-(ioh⁴)⁶Ade], and dihydrozeatin [(ipnoh⁴)⁶Ade] in promoting callus growth of *P. vulgaris* var. Great Northern (A) and *P. lunatus* var. Kingston (B).

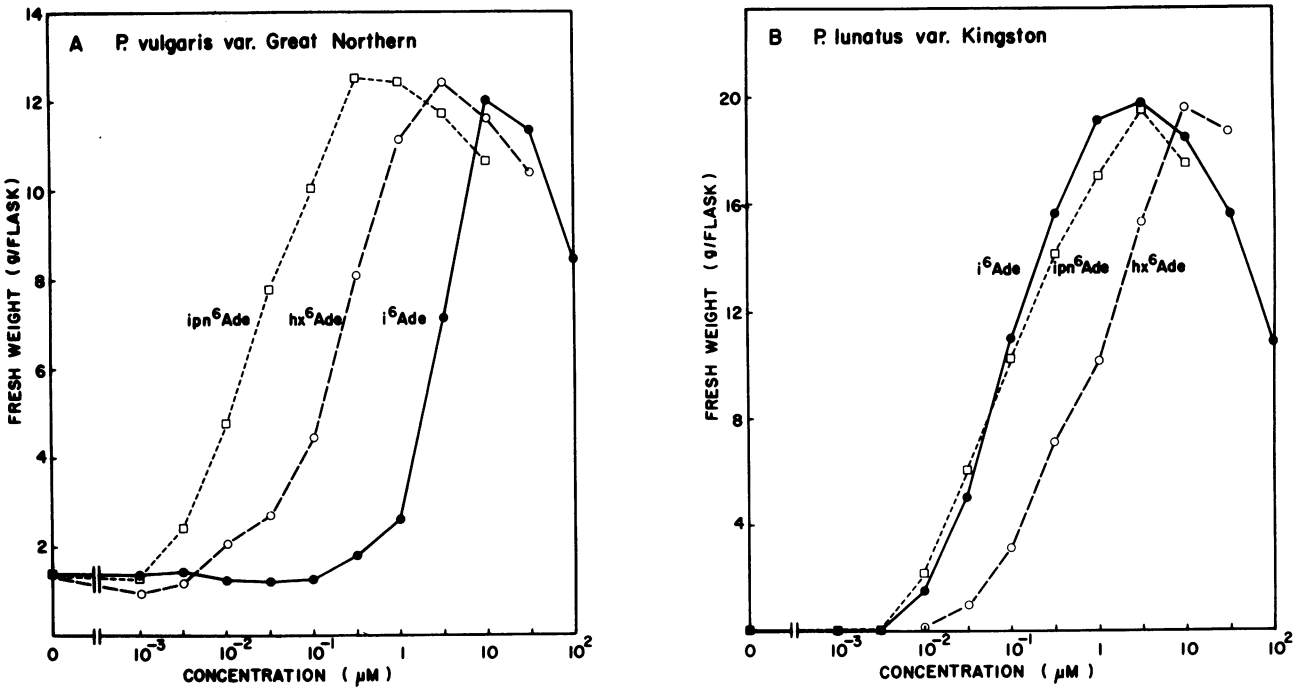


FIG. 3. Cytokinin activities of N^6-(Δ^2-isopentenyl)adenine [i^{6}Ade], N^6-isopentyladenine [ipn^{6}Ade], and N^6-hexyladenine Ade] in promoting callus growth of *P. vulgaris* var. Great Northern (A) and *P. lunatus* var. Kingston (B).

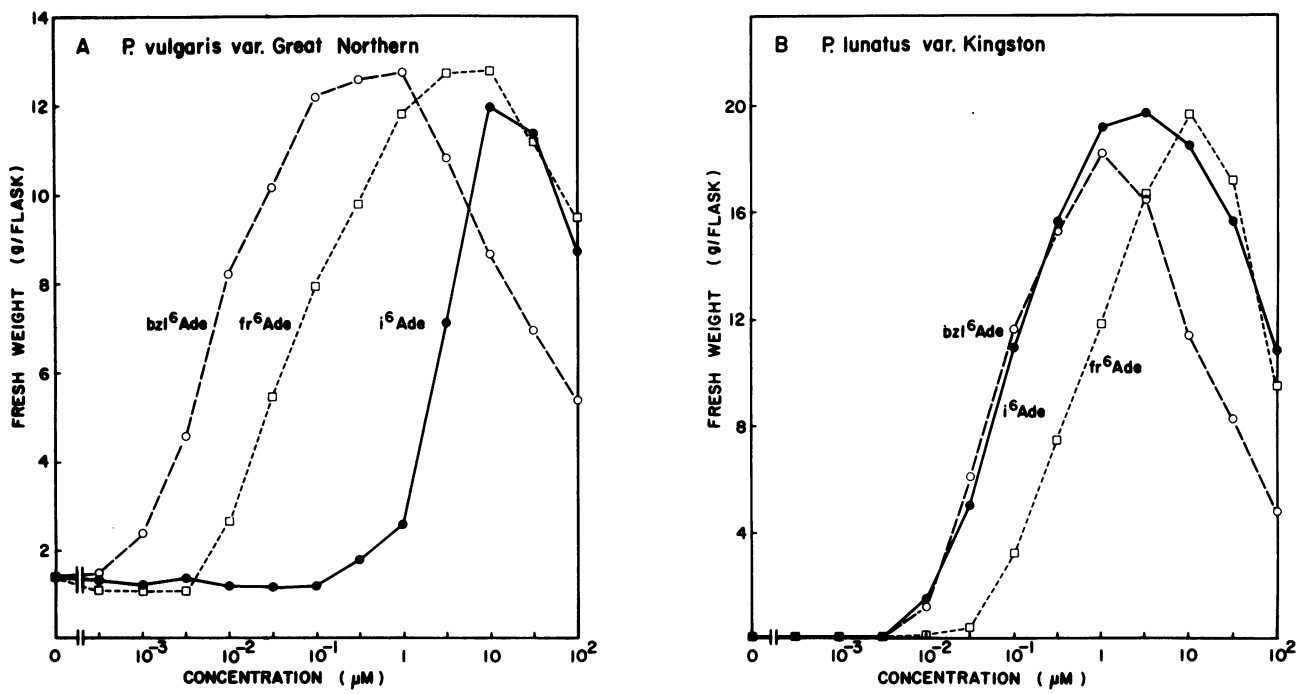


FIG. 4. Cytokinin activities of N^6-benzyladenine [bzl^{6}Ade], kinetin [fr^{6}Ade], and N^6-(Δ^2-isopentenyl)adenine [i^{6}Ade] in promoting callus growth of *P. vulgaris* var. Great Northern (A) and *P. lunatus* var. Kingston (B).

system (3, 4, 8). In *P. vulgaris* var. Great Northern, the presence of the double bond in the N^6 side chain of *trans*-zeatin and N^6-(Δ^2-isopentenyl)adenine results in a dramatic reduction in activity relative to dihydrozeatin and N^6-isopentyladenine. The activities of cytokinins do not appear to be influenced by the auxin source, since the same relative activites of zeatin and dihydrozeatin were obtained when 2,4-D was supplied in place of picloram.

The biochemical basis of the marked difference in growth response of Great Northern and Kingston tissues to cytokinins bearing unsaturated isoprenoid side chains has not yet been investigated. However, Whitty and Hall (12) found that *trans*-zeatin and N^6-(Δ^2-isopentenyl)adenine, as well as their ribosides, were rapidly degraded by a cytokinin oxidase isolated from maize. N^6-Isopentyladenine, on the other hand, was resistant to attack by this enzyme. N^6-Benzyladenine, the most active cytokinin in Great Northern, was also resistant to degradation by the maize enzyme. Therefore, it is conceivable that the observed

differences in cytokinin structure-activity relationships may be related, at least in part, to differences in cytokinin oxidase activity.

The high cytokinin activity exhibited by dihydrozeatin in Great Northern is also of interest in connection with the report that this compound is the major cytokinin present as the free base in fruits of *P. vulgaris* var. Pinto (1). Furthermore, *P. vulgaris* seedlings have been reported to metabolize exogenously supplied *trans*-zeatin to dihydrozeatin (11). This suggests that a correlation may exist between the structural requirements for high cytokinin activity in promoting the growth of *Phaseolus* callus tissue and cytokinin metabolism in intact seedlings. However, comparable data are not available for *P. lunatus*.

Our results demonstrate that pronounced differences in cytokinin structure-activity relationships can be observed in cytokinin-dependent tissue cultures derived from two relatively closely related plant species. If similar differences can be identified between varieties or lines within the same species, the potential would exist for the application of techniques of genetic analysis to investigations of the regulation of cytokinin metabolism and function in plant tissues.

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