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RESPONSES OF *PHASEOLUS LUNATUS* CALLUS TISSUES TO CYTOKININ-ACTIVE UREIDOPURINES

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Key word Index—*Phaseolus lunatus*; Leguminosae; Fabaceae; lima beans; cytokinin structure–activity; ureidopurines; N^6-benzyladenine; Thidiazuron; plant tissue culture.

Abstract—Three ureidopurine derivatives were tested for their ability to promote the cytokinin-dependent growth of callus tissues derived from three genotypes of *Phaseolus lunatus* (cv. Jackson Wonder, cv. Kingston and P.I. 260415). In all three test systems, the ureidopurines exhibited the same relative cytokinin activities (6-phenylureidopurine > 6-*p*-tolylureidopurine > 6-cyclohexylureidopurine) and were less active than the aminopurine derivatives, N^6-benzyladenine and N^6-cyclohexylmethyladenine. Callus tissues of Jackson Wonder, which become cytokinin-autonomous if grown on cytokinin-active urea derivatives, retained cytokinin-dependence on media containing optimal concentrations of ureidopurines. In this respect, the biological activity of the ureidopurines was similar to that of the aminopurine derivatives and differed from that of cytokinin-active urea derivatives tested in this system.

INTRODUCTION

Cytokinin activity is a property of at least two classes of compounds: N^6-substituted aminopurine (adenine) derivatives and certain phenylurea derivatives. Highly active cytokinins may be obtained with either type of structure, but subtle differences in biological properties of the two types of cytokinins are observed in some test systems.

The 6-ureidopurines are compounds that possess structural features of both major classes of cytokinins. The cytokinin activities of 6-ureidopurine derivatives were tested in the tobacco callus bioassay by Skoog *et al.* [1] and in the soybean callus bioassay by Hall *et al.* [2, 3]. The relative activities of particular ureidopurine derivatives were not always equivalent in the two test systems. Thus, 6-phenylureidopurine, which was only weakly active in the soybean callus bioassay, was active at concentrations below 0.1 μM in the tobacco callus bioassay. Chheda and co-workers also tested a number of ureidopurines for biological activity in mammalian cell culture [4, 5] and as inhibitors of adenosine kinase [6]. Although some of these compounds had antiproliferative activity in animal cell cultures, this property was not correlated with cytokinin activity.

The effects of 6-phenylureidopurine and several structurally related compounds on the growth of cytokinin-dependent callus tissues derived from selected genotypes of *Phaseolus lunatus* have been examined in the work reported here. Ureidopurine derivatives had not previously been tested in *P. lunatus* cultures, and it was of interest to assess the cytokinin activity of these compounds in callus tissues from this plant source and to determine whether genotypic variation in sensitivity to ureidopurines might be present in the species. In addition, the effects of ureidopurine derivatives on callus tissues of *P. lunatus* cv. Jackson Wonder were of special interest, because callus tissues of this genotype exhibit biological responses to cytokinin-active adenine derivatives that are somewhat different from those elicited by cytokinin-active urea derivatives [7].

RESULTS AND DISCUSSION

Three genotypes of *P. lunatus* (cv. Jackson Wonder, P. I. 260415 and cv. Kingston) were selected for the present study. Callus tissues of all three genotypes can be maintained as cytokinin-dependent lines when grown on optimal concentrations of cytokinin-active purine (adenine) derivatives [8], and callus tissues derived from P. I. 260415 remain cytokinin-dependent under all conditions tested to date [7, 8]. However, Capelle *et al.* [7] found that callus tissues of Jackson Wonder became cytokinin-autonomous when grown on cytokinin-active urea derivatives or on less than optimal concentrations of cytokinin-active purine derivatives. Thus, the urea derivative thidiazuron (*N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea), although highly active in promoting the growth of callus tissues of this genotype [9], was ineffective in preventing transformation to a cytokinin-autonomous growth habit. The properties of callus tissues of cv. Kingston appear intermediate between those of the other two genotypes [8].

The cytokinin activities of three ureidopurine derivatives (6-phenylureidopurine, PheNC^6Ade; 6-cyclohexylureidopurine, hPheNC^6Ade; and 6-*p*-tolylureidopurine, TolNC^6Ade) and two aminopurine derivatives (N^6-benzyladenine, b^{6}Ade; and N^6-cyclohexylmethyladenine, hb^{6}Ade) were tested on callus tissues derived from the three *P. lunatus* genotypes. Structures of the test compounds are shown in Fig. 1. The abilities of the five

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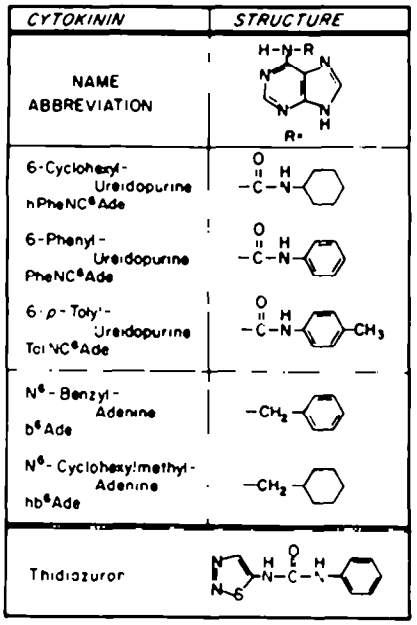

Fig. 1. Structures of cytokinins tested for ability to promote the growth of *Phaseolus lunatus* callus tissues.

compounds to promote the growth of callus tissues of *P. lunatus* cv. Jackson Wonder are compared in Fig. 2, and the results of similar experiments using callus tissues of cv. Kingston and P. I. 260415 are shown in Figs 3 and 4, respectively. In all three test systems, the two aminopurine derivatives (b^6Ade and hb^6Ade) were more active than any of the ureidopurine derivatives. The cytokinin activity of

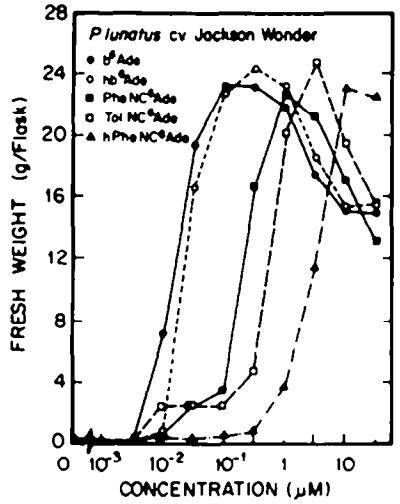

Fig. 2. Cytokinin activities of ureidopurine and aminopurine derivatives in the promotion of growth of callus cultures of *Phaseolus lunatus* cv. Jackson Wonder. Stock cultures of callus tissue, obtained as described in Experimental, were transferred to media containing the test compounds at the concentrations indicated. The average fresh weights per flask were determined after a growth period of 5 weeks. Abbreviations are defined in Fig. 1.

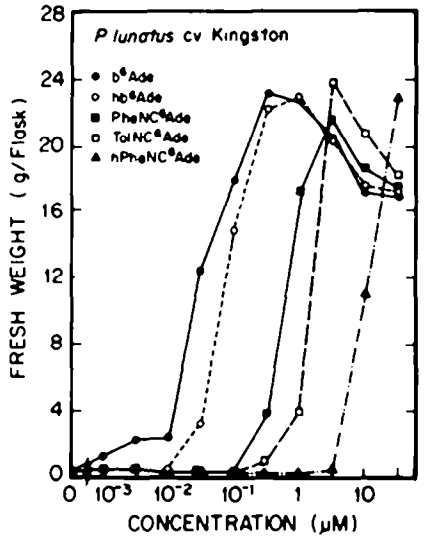

Fig. 3. Cytokinin activities of ureidopurine and aminopurine derivatives in the promotion of growth of callus cultures of *Phaseolus lunatus* cv. Kingston. Stock cultures of callus tissue, obtained as described in Experimental, were transferred to media containing the test compounds at the concentrations indicated. The average fresh weights per flask were determined after a growth period of 5 weeks. Abbreviations are defined in Fig. 1.

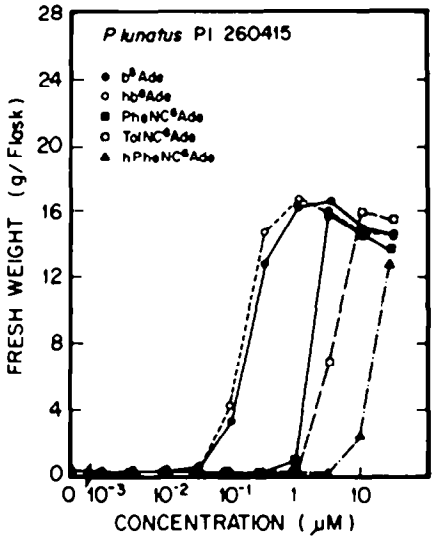

Fig. 4. Cytokinin activities of ureidopurine and aminopurine derivatives in the promotion of growth of callus cultures of *Phaseolus lunatus* P.I. 260415. Stock cultures of callus tissue, obtained as described in Experimental, were transferred to media containing the test compounds at the concentrations indicated. The average fresh weights per flask were determined after a growth period of 5 weeks. Abbreviations are defined in Fig. 1.

hb^6Ade, which had not previously been tested with these tissues, was approximately equivalent to that of b^6Ade in all of the *P. lunatus* callus tissues examined here. The three ureidopurine derivatives exhibited the same relative activities in all three tissues (Phe*NC*^{6}Ade > Tol*NC*^{6}Ade

> hPheNC^6Ade). In contrast to the results obtained with the aminopurine derivatives, reduction of the aromatic ring of the side chain of PheNC^6Ade (to give hPheNC^6Ade) resulted in considerable loss of cytokinin activity in tests with these tissues. It is also of interest that PheNC^6Ade appears to be a moderately active cytokinin in promoting the growth of *P. lunatus* callus tissues. This contrasts with the reported low activity of this compound in tests using soybean callus tissue [3].

Although no genotypic differences were observed in the order of activities of the test compounds, callus tissues of the three *P. lunatus* cultivars differed in the overall sensitivity of their responses to cytokinins. Thus, callus tissues of Jackson Wonder responded to low levels of the test compounds; callus tissues of cv. Kingston were intermediate in sensitivity; and callus tissues of P. I. 260415 required relatively high levels of cytokinin to support growth. For example, the concentration of PheNC^6Ade calculated to be required to give one-half maximal growth varied from 0.22 μM in Jackson Wonder to 0.71 μM in Kingston to 1.6 μM in P. I. 260415 callus tissues.

The ability of the ureidopurine derivatives to suppress the development of cytokinin autonomy in callus tissues of Jackson Wonder was also tested. Tissues of Jackson Wonder were grown on optimal concentrations of the ureidopurines and then transferred to cytokinin-free media. Similar transfers performed with tissues grown on optimal concentrations of the two aminopurine derivatives and the cytokinin-active urea derivative, Thidiazuron, were included as controls. The results are shown in Table 1. As expected, Jackson Wonder tissue grown on media containing thidiazuron as the cytokinin source displayed a cytokinin-autonomous growth habit on transfer to cytokinin-free media, while tissues grown on optimal concentrations of the aminopurine derivatives remained cytokinin-dependent. The Jackson Wonder tissue grown on optimal concentrations of the ureidopurine derivatives also remained cytokinin-dependent. Although there appeared to be a slight increase in the background growth observed on cytokinin-free medium after exposure of callus tissues to the two less active ureidopurine derivatives (hPheNC^6Ade and TolNC^6Ade), the behaviour of these callus tissues was clearly distinct from that of Thidiazuron-treated tissues and closely resembled that of tissues grown on cytokinin-active aminopurines. Thus, the ureidopurine derivatives appear similar to the cytokinin-active aminopurine derivatives in their ability to suppress the development of cytokinin autonomy in tissues of *P. lunatus* cv. Jackson Wonder.

EXPERIMENTAL

Chemicals. Samples of 6-phenylureidopurine and 6-*p*-tolylureidopurine were kindly provided by F. Skoog (Institute of Plant Development, University of Wisconsin-Madison). Thidiazuron (*N*-phenyl-*N'*-1,2,3-thidiazol-5-ylurea) was a gift from Schering AG. N^6-Cyclohexylmethyladenine was synthesized in this laboratory by reacting cyclohexylmethylamine with 6-chloropurine. All other chemicals were purchased from commercial sources.

Plant materials. Seeds of *P. lunatus* cv. Jackson Wonder were obtained from Asgrow Seed Co. Seeds of cv. Kingston were obtained locally. Seeds of P. I. 2160415 were provided by the Regional Plant Introduction Station, Washington State University, Pullman, Washington.

Tissue culture medium. The basal medium used to culture *Phaseolus* callus tissues consisted of the inorganic nutrients of Murashige and Skoog [10] with the following organic substances added: *myo*-inositol (100 mg/l.), thiamine-HCl (1 mg/l.), nicotinic acid (5 mg/l.), pyridoxine-HCl (0.5 mg/l.), sucrose (30 g/l.) and picloram (2.5 μM). The pH of the medium was adjusted to 5.7 and Difco Bacto-Agar (10 g/l.) was added. The medium was dispensed

Table 1. Effects of ureidopurines and other cytokinin-active compounds on the development of cytokinin autonomy in callus tissues of *Phaseolus lunatus* cv. Jackson Wonder

Cytokinin*	Cytokinin concentration required for optimal growth (μM)	Average fresh weight (g/flask)	
		Cytokinin-containing medium (second passage)	Cytokinin-free medium (third passage)
hPheNC^6Ade	10.0	23.0	0.5
PheNC^6Ade	1.0	22.7	0.1
TolNC^6Ade	3.0	24.7	0.3
Thidiazuron	0.01	20.4	7.2
b^{6}Ade	0.3	23.2	0.1
hb^{6}Ade	0.3	24.3	0.1

Callus tissues of *P. lunatus* cv. Jackson Wonder (grown as described in Experimental) were transferred to media containing optimal concentrations of the indicated cytokinins for one passage and then to cytokinin-free media. Fresh weight values were determined after growth periods of 5 weeks.

*b^{6}Ade = N^6-benzyladenine, hb^{6}Ade = N^6-cyclohexylmethyladenine, hPheNC^6Ade = 6-cyclohexylureidopurine, PheNC^6Ade = 6-phenylureidopurine, TolNC^6Ade = 6-*p*-tolylureidopurine, thidiazuron = *N*-phenyl-*N'*-1,2,3-thidiazol-5-ylurea.

into 125 ml conical flasks (50 ml per flask) and autoclaved at 120° for 15 min. For callus initiation and the maintenance of stock cultures, kinetin (5 μM) was added to the basal medium. In tests of cytokinin activity, appropriate amounts of the test compounds were dissolved in dimethylsulphoxide and added to the autoclaved tissue culture flasks prior to solidification of the medium [11]. The final concn of dimethylsulphoxide in the tissue culture medium was 0.025 ml per flask containing 50 ml medium.

Growth and harvest of Phaseolus *callus tissues.* Tissue cultures were established from the hypocotyl tissue of 5-day-old seedlings as described in ref. [12]. Four replicate callus lines derived from four different seedlings were established for each genotype. The callus tissue that formed on the initial explants was transferred once (first passage) on the same medium used for callus initiation. Tests for responses to cytokinins were performed in the second passage of the callus tissue using 3-week-old first passage cultures as stock tissue. Tests for cytokinin autonomy were performed by transferring 4-week-old second passage tissues to cytokinin-free media. In all cases, three pieces of callus tissue, weighing *ca* 25 mg each, were planted per flask. Tissues were harvested after a 5-week growth period. Fresh weight values are the averages of four replicate flasks (corresponding to the 4 replicate lines of callus tissue established for each genotype).

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