CYTOKININ PRODUCTION BY TOMATO ROOT: NUTRITIONAL AND HORMONAL FACTORS AFFECTING THE AMOUNT OF CYTOKININ RELEASED FROM THE ROOTS

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Introduction

The nutritional status of a plant, in particular the level of the nitrogen source, has been known to affect cytokinin production by the roots of the plant. Reduced amounts of cytokinin were found in the root exudate and the root extract of sunflower[20] and in the root extract of *Solanum andigena*[23], when these plants were grown in media containing low levels of nitrogen. YOSHIDA and ORITANI[24] reported that nitrogen top-dressing given to rice plants increased the amount of cytokinin in the root exudate.

There are many reports which suggest that the production of one kind of growth regulator is under the control of another one. For example, the application of a cytokinin to different plant systems resulted in an increase in the level of extractable auxin[8,9,16]. However, the effects of growth regulators on cytokinin production by the root system have not yet been investigated.

We have previously reported that cytokinins, predominantly a substance co-chromatographed with zeatin riboside, were released from cultured tomato roots into the medium and that the amount of cytokinin which accumulated in the medium after a 7-day-culture was about 8 times that in the root tissue itself[10]. The amount of cytokinin released from the roots appears to mirror that produced in the root tips. Therefore, the root tip culture may provide a suitable system to investigate what kind of substances affected cytokinin production by root tips.

Herein we report on some of the nutritional and hormonal factors which affect cytokinin production by tomato root tips using the root tip

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culture method.

Materials and Methods

Tomato seeds (*Lycopersicon esculentum* MILL. cv. HAGOROMO) were sterilized with 0.4% sodium hypochrolite solution for 30 min, rinsed three times with sterile water and allowed to germinate at 25°C in the dark in modified White's basal medium[10]. After 7 days, root tips about 1 cm long (each ca. 1.5 mg in fresh weight) were excised from the seedlings. Three fragments of the tips were then transferred to a 100 ml Erlenmeyer flask containing 10 ml basal medium supplemented with various additions and cultured under the same condition as stated above for 7 days. At the end of each culture, the roots were removed from the medium by filtration. The medium (400 ml for each determination) was adjusted to pH 2.5 with 1 N HCl and then passed through a Dowex 50W-X4 (50-100 mesh) cation exchange column of 50 ml volume. The column was washed with 500 ml distilled water, and the adsorped substances were eluted with 3 N NH_4OH. To avoid heat decomposition of cytokinins, the column was cooled with ice-cold water until all the resin had been converted to the NH_4^+ from. The eluate was evaporated to dryness below 40°C, and the resulting residue was chromatographed on papers with iso-propanol : ammonia : water (10 : 1 : 1 v/v). After complete drying, the chromatograms were cut into 10 equal Rf strips, and each of them was placed directly into a bioassay medium for cytokinin. Cytokinin activities were determined using soybean callus bioassay[11]. Two peaks of cytokinin activity were always found on the chromatograms at Rf 0-0.1 and 0.5-0.7. The fast-moving activity, which co-chromatographed with zeatin and its riboside, was predominant. Therefore, the amount of cytokinin in each medium was calculated from the activities recorded at Rf 0.5-0.7 on the chromatograms using as a reference the growth curve for authentic zeatin.

It is possible that basic or amphoteric compounds supplemented to the medium remained in the cytokinin fraction and thereby affected soybean callus growth. In preliminary experiments, it was found that every such compound used in this study migrated below Rf 0.5 on the paper chromatograms. Furthermore, such compounds at a concentration of 0.1 mM exerted no appreciable effects on the soybean callus growth induced by 10 nM zeatin. Thus the growth of soybean callus observed at Rf 0.5-0.7 appeared to represent the amount of cytokinin. As practical difficulties did not permit sufficient replications of each experiment to allow statistical analysis of individual results, the control experiment (no additions) was repeated 5 times and

the significant differences from the controls were calculated.

Results

Inorganic nitrogen compounds and amino acids

At first, the effects of various nitrogen compounds on the amount of cytokinin released from the roots into the medium and on the root growth were examined. The root growth was measured by the increase of fresh weight. The dry weight of the roots was always 7.9±0.35% (±SD, $n=20$) of the fresh weight. White's basal medium itself contains 3.2 mM nitrate as nitrogen source. The results are shown in Table 1. The addition of 2 mM NH_4Cl doubled the amount of cytokinin, whereas the addition of KNO_3 and urea at the same concentration was only slightly effective in increasing

TABLE 1. Effects of inorganic nitrogen compounds and amino acids on the amount of cytokinin released from cultured tomato roots and the root growth. Tomato root tips were cultured in White's medium supplemented with each compounds for 7 days. The amount of cytokinin in each medium was calculated from the growth of soybean callus recorded at Rf 0.5-0.7 on the paper chromatogram developed with iso-propanol : ammonia : water (10 : 1 : 1 v/v) using as a reference the growth curve for authentic zeatin. The result of the control is expressed as the average of the results of 5 different experiments±SE

Addition		Cytokinin in medium		Root growth	
	(mM)	Zeatin equiv. (ng/l)	% of control	fr. wt. (mg/root)	% of control
None		75±5.9	100	19.6±1.0	100
NH_4Cl	2	160*	213*	21.9	112
KNO_3	2	88	117	31.4*	160*
Urea	2	83	111	2.4*	12*
L-Asp	0.2	360*	480*	35.4*	181*
L-Glu	0.2	200*	267*	30.3*	155*
L-Asp+L-Glu	0.2 each	123*	164*	19.4	99
L-Asn	0.2	91	121	28.8*	144*
L-Gln	0.2	98	131	24.4	124
L-Lys	0.2	87	116	30.8*	157*
L-Arg	0.2	56	75	25.1*	128*
L-Leu	0.2	42*	56*	13.6*	69*

* Differences are significant from the control at 1% level.

the amount. Applications of various amino acids at a concentration of 0.2 mM turned out miscellaneous results. Among the amino acid tested, L-aspartate was most effective in increasing the amount of cytokinin followed by L-glutamate. The amides of them increased the amount slightly. L-lysine did not affected the amount, and L-arginine and L-leucine were inhibitory.

Another interesting finding was that there was no causal relationship between the amount of cytokinin released from the roots and the root growth. For example, KNO_3 and urea, both of which induced a slight increase in the amount of cytokinin, showed different effects on the root growth, the former being stimulative while the latter severely inhibitory.

The effect of glycine was separately investigated because White's medium contains 0.4 mM glycine. The addition of glycine to a glycine-free medium at concentrations of 0.2, 0.4, 0.8 and 1.2 mM caused a slight increase in the amount of cytokinin released from the roots but a decrease in the root

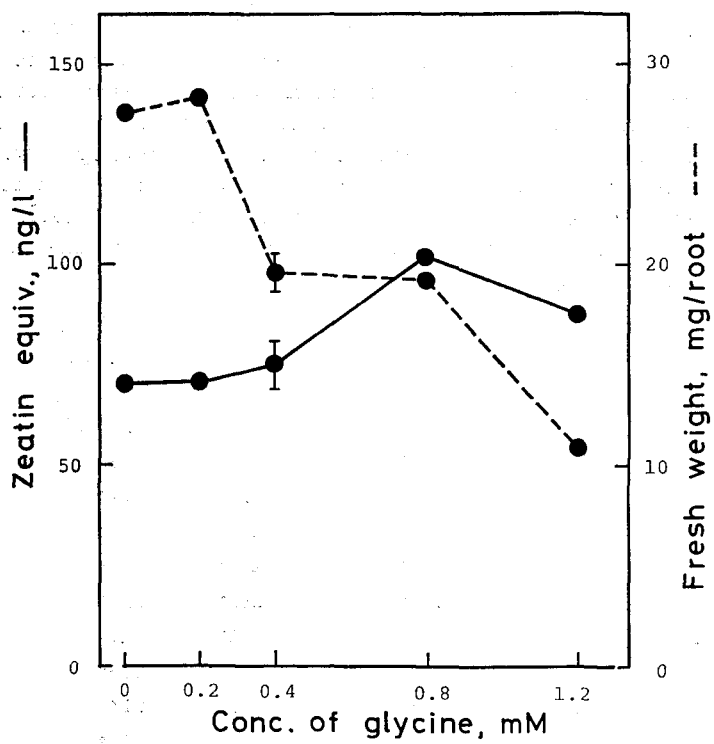

Fig. 1. Effect of glycine on the amount of cytokinin released from cultured tomato roots and the root growth. Tomato root tips were cultured in glycine-free White's medium supplemented with glycine at various concentration for 7 days. Details are the same as those in Table 1.

growth with increasing concentrations (Fig. 1).

Purines and mevalonic acid

Various purines were added to the medium at a concentration of 0.2 mM. 5′-AMP, 5′-IMP and adenine were not effective in increasing the amount of cytokinin (Table 2). Adenosine was slightly effective. Unexpectedly, applications of degradative products of adenylate: inosine and hypoxanthine, caused a considrable increase in the amount of cytokinin. In contrast, xanthine, which is a degradative product of hypoxanthine, showed little effect.

Mevalonic acid (lactone form, 0.2 mM), which is a precursor of the isopentenyl side chain of cytokinin[1,4,12] exerted no effect on the amount of cytokinin even when applied together with 5′-AMP.

TABLE 2. Effects of purines and mevalonic acid on the amount of cytokinin released from cultured tomato roots and the root growth. Tomato root tips were cultured in White's medium supplemented with each compounds for 7 days. Details are the same as those in Table 1

Addition		Cytokinin in medium		Root growth	
	(mM)	Zeatin equiv. (ng/l)	% of control	fr. wt. (mg/root)	% of control
None		75±5.9	100	19.6±1.0	100
5′-AMP	0.2	72	96	39.0*	199*
Adenosine	0.2	118*	157*	21.7	111
Adenine	0.2	73	97	22.5	115
5′-IMP	0.2	79	105	29.8*	152*
Inosine	0.2	164*	219*	11.9*	61*
Hypoxanthine	0.2	223*	297*	28.4*	145*
Xanthine	0.2	116*	154*	16.3	83
Mevalonic acid (MVA)	0.2	72	96	30.3*	153*
MVA+5′-AMP	0.2 each	72	100	24.0	122

Growth regulators

Effects of various concentrations of growth regulators: indole-3-acetic acid (IAA), gibberellic acid (GA_3) and abscisic acid (ABA), on the amount of cytokinin released from the roots and the root growth are shown in Fig. 2. IAA showed no appreciable effect on the amount of cytokinin at concentrations up to 20nM, but above that level it was severely inhibitory. The

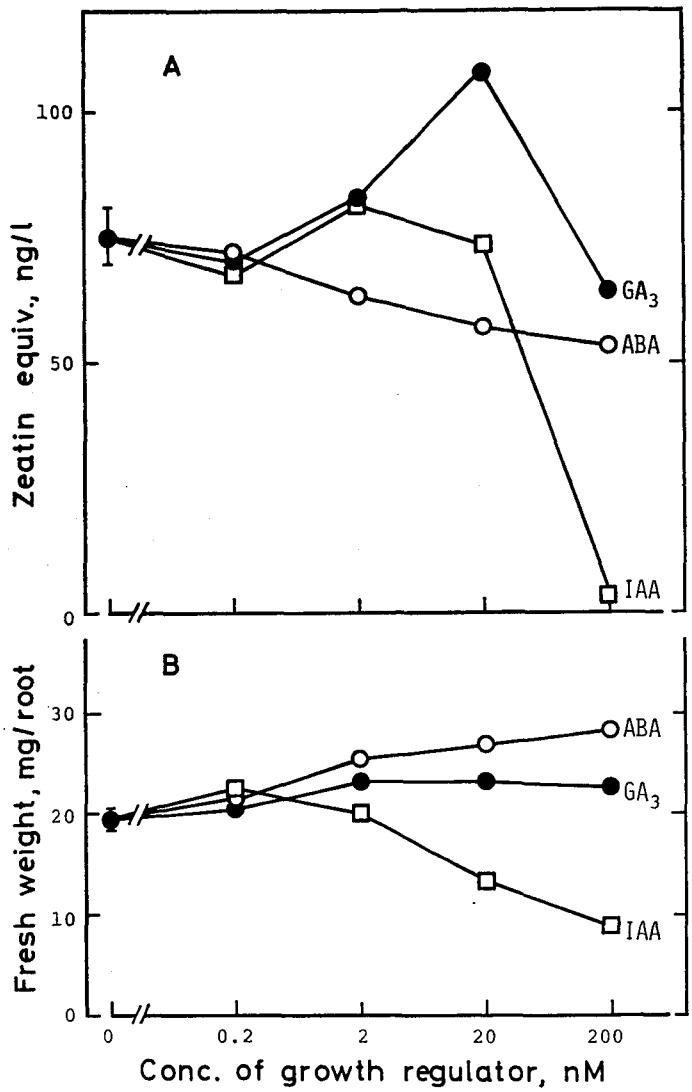

Fig. 2. Effects of growth regulators on the amount of cytokinin released from cultured tomato roots (A) and the root growth (B). Tomato root tips were cultured in White's medium supplemented with each growth regulator at different concentrations for 7 days. Details are the same as those in Table 1.

growth of the roots was suppressed by IAA above 2 nM. No signs of callus induction by IAA were observed at any of the concentrations. Raising the ABA concentration caused a progressive decrease in the amount of cytokinin

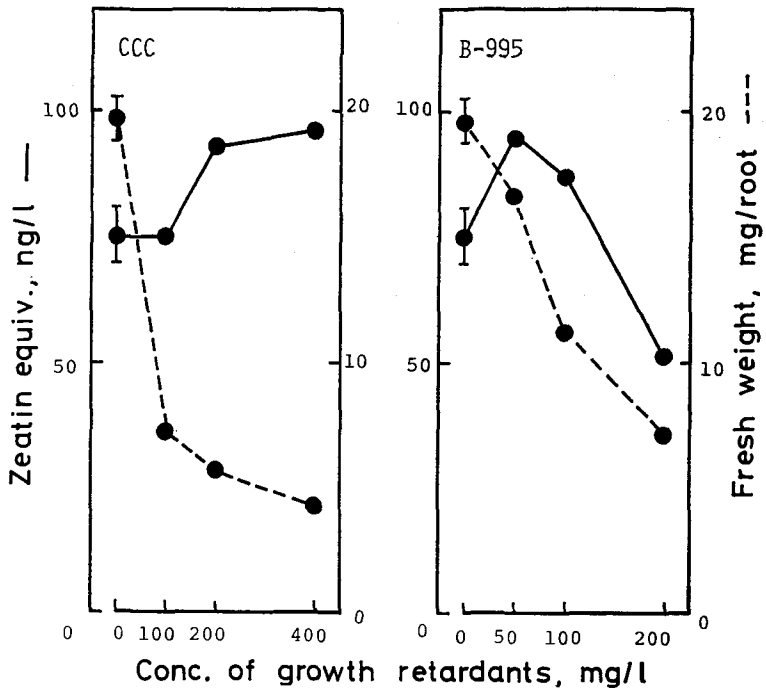

Fig. 3. Effects of CCC and B-995 on the amount of cytokinin released from cultured tomato roots and the root growth. Tomato root tips were cultured in White's medium supplemented with each growth retardant at different concentrations for 7 days. Details are the same as those in Table 1.

but enhanced the root growth slightly. GA_3 at 20 nM was effective in increasing the amount of cytokinin. The root growth was not affected by GA_3 at any of the concentrations.

Growth retardants

SKENE[18] reported that (2-chloroethyl)trimethylammonium chloride (CCC), an inhibitor of gibberellin biosynthesis[19], increased the level of cytokinin in the xylem sap of grape vine. In the next experiment, the effects of growth retardants: CCC and succinic acid 2, 2-dimethylhydrazide (B-995), were examined. CCC caused a slight increase in the amount of cytokinin released from the roots but markedly inhibited the root growth (Fig. 3). B-995 was also stimulative in increasing the cytokinin at a low concentration but inhibitory to the root growth.

Discussion

The amount of cytokinin released from the cultured tomato roots into the medium was influenced by various nutritional and hormonal additions to the medium. The amount seems to be regulated by both the rate of cytokinin biosynthesis and degradation. It is unlikely that the amount was regulated by the rate of cytokinin release from the roots, because the cytokinin pool size in the root tissue was very small[10]. Therefore, the amount of cytokinin released from the roots seems to represent that 'produced' by them, which is the balance between synthesis and degradation.

The application of NH_4Cl is known to promote cytokinin accumulation in cultured tissues such as soybean root callus[17] and *Vinca* crown gall tumor[15], both of which are capable of synthesizing cytokinins. Our present results also indicated that certain compounds containing reduced nitrogen: NH_4Cl, L-aspartate and L-glutamate, were able to increase the amount of cytokinin released from the cultured tomato roots (Table 1). Adenine and adenosine have been reported as precursors of zeatin and its riboside[2,5,6,14,15]. Both aspartate and glutamate are involved in purine biosynthesis[3], and an application of NH_4Cl yields a higher concentration of adenine nucleotide in the roots of soybean and sunflower than is found with KNO_3[22]. This increase in the amount of cytokinin released from the roots may be due to stimulation of cytokinin biosynthesis.

Applications of various purines led to some conflicting results (Table 2). Ineffectiveness of some purines on the cytokinin release from the roots seems to be due to various characteristics of the purines such as their inability to incorporate into cells and their susceptibility to degradating enzymes. When the results are arranged in the sequence of the main degradation pathway of adenylate: 5'-AMP, adenosine, inosine and hypoxanthine, the order of activity in increasing the cytokinin shows the reverse relationship to the sequence. Although it is tempting to speculate that hypoxanthine is a main precursor for zeatin biosynthesis, it is more likely that the effect of hypoxanthine is caused by inhibition of cytokinin degradation.

IAA severely reduced the amount of cytokinin released from the roots (Fig. 2). This result may indicate that in intact plants the amount of cytokinin translacated out of the roots is reduced by IAA produced in the shoot apex. Some reports[9,16] have suggested that root produced cytokinin stimulates auxin production in the shoot apex. Well-balanced growth of plants may be achieved by stimulation of auxin production by cytokinin and inhibition of cytokinin production by auxin which occur in the shoot apex and

root tips respectively.

Recently, Van STADEN and DIMALLA[19] reported that spray applications of GA_3 to *Bougainvillea* plants caused an increase in endogenous cytokinin level in the root tissues. In this investigation, applications of GA_3 and growth retardants induced a slight increase in the amount of cytokinin released from the tomato roots. These results suggest a close relation between gibberellin and cytokinin biosynthesis.

No causal relationship was found between the amount of cytokinin released from the roots and the root growth; this finding is in agreement with that reported by PETERSON and MILLER[15] in *Vinca* crown gall tumor tissue. They found that cytokinin content in the tissue is independent on its growth. The root growth is brought about by cell division at their meristematic regions. Therefore, this finding seems to exclude the possibility proposed by GOLDACRE[7] that cytokinin production by roots is a normal accompaniment of cell division at their meristematic regions.

Summary

Applications of various compounds into a culture medium in which excised tomato root tips (*Lycopersicon esculentum* MILL.) were grown affected the amount of cytokinin released from the roots into the medium. Several compounds containing reduced nitrogen increased the amount. Among the amino acids tested, L-aspartate was found to be the most effective followed by L-glutamate. Applications of various purines revealed that hypoxanthine was the most effective followed by inosine. Growth regulators also influenced the amount. Indole-3-acetic acid severely decreased the amount while gibberellic acid increased it slightly.

No causal relationship was found between the amount of cytokinin released from the roots and the root growth. This result suggests that the cytokinin production by root tips is not a normal accompaniment of cell division at their meristematic regions.

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