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Effect of *in vivo* and *in vitro* application of the cytokinin N^6-(*m*-hydroxybenzyl)adenosine on respiration and membrane transport processes in sugar beet

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Abstract

Sugar beet grown in pots was sprayed with N^6-(*m*-hydroxybenzyl)adenosine, (*m*OH)-[9R]BAP, one of the synthetic cytokinins. Root tissue was then examined for respiration and for H^+-adenosinetriphosphatase activity and both leaf and root tissue served as the object for 6-deoxy-D-glucose and 2-aminoisobutyric acid uptake estimations. Treatment with (*m*OH)[9R]BAP depressed the uptake of oxygen by the roots of both young and old plants by 17 - 30 % while addition of (*m*OH)[9R]BAP to the respiring slices decreased it by 10 - 23 %. Uptake of 6-deoxy-D-glucose was mostly diminished by *in vivo* spraying with the cytokinin (by up to 12 % in leaves and by up to 60 % in roots), as well as by adding it to the experimental vessel (insignificantly in the leaves but by up to 80 % in the roots). The H^+-ATPase activity was stimulated both *in vivo* and *in vitro* appreciably in young plants but not at all in plants at the end of their vegetation period.

Additional key words: *Beta vulgaris*, H^+-ATPase.

Introduction

Various cytokinins have been shown to interact in a number of metabolic reactions and growth processes of plants; typically, the classical 6-benzylaminopurine affected the alternative respiratory pathway in soybean (Musgrave *et al.* 1987), respiration of soybean mitochondria (Miller 1979, 1982) and its complexes with Cu^{II} mimicked the action of superoxide dismutase in plant senescence (Inoue and Hirobe 1986). At the same time, cytokinins were reported to favourably influence the rate of photosynthesis and accumulation of starch (Luštinec *et al.* 1984, Chernyadev *et al.*

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1986, Musgrave 1994, Synková *et al.* 1996). The effects of natural cytokinins are mimicked by a variety of synthetic ones (*e.g.*, Vaňková *et al.* 1992).

In the context of a programme targetted at increasing the sugar yield of sugar beet and at increasing its quality, several cytokinins, among them N^6-(*m*-hydroxybenzyl)-adenosine, (*m*OH)[9R]BAP, and a variety of features (growth, sugar content, photosynthetic activity, *etc.*) were evaluated. The present report concerns one section of the project, viz. several *in vitro* assays, including respiration of root tissue, uptake of sugars and amino acids by leaf and root tissue, and acidification of the external medium by thin root slices.

Materials and methods

Sugar beet (*Beta vulgaris* L., cv. Edda) was sown in 12-dm^3 pots in mid-April. Cytokinin (*m*OH)[9R]BAP, which exhibits the same biological activity as *trans*-zeatin (Kamínek *et al.* 1987) was applied by spraying (3 μM in 0.03 % ethanol, together with the surfactant *Cytovet* at 80 mm^3 dm^{-3}) to plants after 10 and 20 weeks of vegetation. Controls were sprayed with the same solution without the cytokinin. All the tests described below were carried out 10 - 15 d after the spraying.

Respiration: Classical manometry in a Warburg apparatus was used to estimate the uptake of oxygen by root slices, roughly 8 × 8 × 0.5 mm in size. The slices were cut from the upper part of the root, just below the soil surface but not the green part.

Acidification of the external medium: Here again slices from the upper non-green part of the root were used to monitor pH changes on a *Cole-Parmer* model *59003* pH-meter with a combination glass-calomel electrode.

Uptake of nonelectrolytes: Leaf segments without petioles about 8 × 8 cm in size were suspended in 2 cm^3 of an aqueous solution of either ^{3}H-6-deoxy-D-glucose (quinovose), or ^{14}C-D-xylose (at 0.1 mM concentration) and incubated at 25 °C for 30, 60 and 120 min. They were then removed, rinsed with distilled water and placed in a liquid scintillation cocktail. Radioactivity was measured in a *Beckman* scintillation counter and corrections were taken for both quenching and fluorescence in the samples.

Chemicals: The synthetic cytokinin N^6-(*m*-hydroxybenzyl)adenosine was obtained from Dr. T. Vaněk, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague. 6-Deoxy-D-glucose and erythrosin B were purchased from *Sigma* (St. Louis, USA), 2-aminoisobutyric acid was from *Aldrich* (Beerse, Belgium). Fusicoccin was a gift from Prof. U. Lüttge of the Darmstadt Technical University. All other nonlabelled chemicals were from *Lachema* (Brno, Czech Republic) and were of the highest purity available.

Universally labelled ^{3}H-6-deoxy-D-glucose was custom-made at the then Institute of Nuclear Biology and Radiochemistry of the Czechoslovak Academy of Sciences (cf. Kotyk *et al.* 1975) and was checked for purity before the experiments.

Universally labelled ^{14}C-D-xylose was from *Amersham International* (Amersham, UK).

Results and discussion

Respiration: The oxygen uptake rate by the differently treated sugar-beet root tissue is shown in Table 1. The values for the controls tally very nicely with those published by Zahradníček *et al.* (1980).

Both the acute effect of (*m*OH)[9R]BAP and the one exerted on plants in pots are clearly visible (cf. Miller 1979). In practical terms this means that losses of sugar through endogenous respiration (mainly of sucrose) are substantially diminished by the application of (*m*OH)[9R]BAP.

Table 1. Oxygen uptake rate [$mm^3(O_2)\ g^{-1}$(d.m.) s^{-1}] and mean inhibition [%] of sugar-beet root after different treatments with the synthetic cytokinin (*m*OH)[9R]BAP.

Variant	Addition	Age	Oxygen uptake	Inhibition
Control		12 weeks	10.8 - 11.5	-
	50 μM (*m*OH)-[9R]BAP	12 weeks	10.0 - 10.2	10
Sprayed after 10 weeks		12 weeks	7.0 - 8.0	33
	50 μM (*m*OH)-[9R]BAP	12 weeks	6.8 - 7.2	37
Control		22 weeks	9.2 - 9.9	-
	50 μM (*m*OH)-[9R]BAP	22 weeks	7.3 - 8.9	15
Sprayed after 10 and 20 weeks		22 weeks	6.5 - 9.4	17
	50 μM (*m*OH)-[9R]BAP	22 weeks	4.7 - 6.7	40

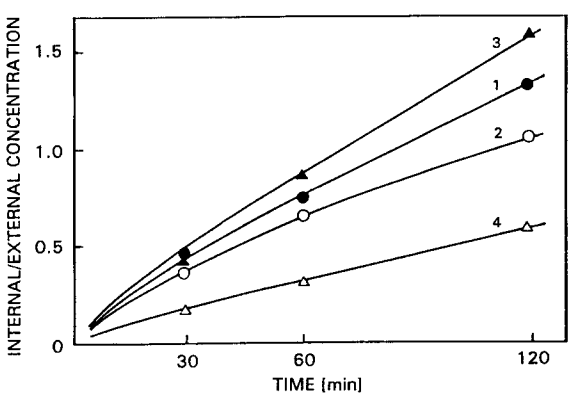

Fig. 1. Uptake of labelled 0.1 mM 6-deoxy-D-glucose shown as the ratio of intracellular to extracellular concentration by leaves of untreated (*curve 1*) and (*m*OH)[9R]BAP-treated (*curve 2*) plants, and by roots of untreated (*curve 3*) and (*m*OH)[9R]BAP-treated (*curve 4*) plants. Plants were 12 weeks old and, where indicated, after a single (*m*OH)[9R]BAP spraying done two weeks before the experiment.

Uptake of 6-deoxy-D-glucose: In young plants the leaf tissue took up this sugar (a nonmetabolized analogue of D-glucose) relatively slowly and almost linearly over the entire incubation period of 2 h. The same may be said about the uptake by the root tissue (Fig. 1). Clearly, previous treatment with the cytokinin invariably caused a slower uptake of the sugar. With older plants, after two sprayings with the cytokinin, the pattern was qualitatively the same but uptake by the leaf tissue was substantially slower than in the young plants while roots took it up with the same avidity (Table 2). The addition of 50 μM (*m*OH)[9R]BAP to leaf or root slices had a less pronounced depressive effect on the uptake than did application of the substance to living plants; in fact, in the leaves it even increased the uptake of the sugar in three of the four variants examined.

Table 2. Uptake of 0.1 mM 6-deoxy-D-glucose by sugar-beet leaves and roots after (*m*OH)[9R]BAP treatment. R stands for the ratio of internal-to-external concentration of the sugar after a 1-h incubation (means of three independent measurements).

Tissue	Spraying	Addition of 50 μM (*m*OH)[9R]BAP	R
Leaf (young)	none	–	1.25
		+	1.68
	one	–	1.10
		+	0.89
Leaf (old)	none	–	0.75
		+	0.75
	two	–	0.50
		+	0.58
Root (young)	none	–	1.52
		+	0.60
	one	–	0.62
		+	0.52
Root (old)	none	–	1.74
		+	1.34
	two	–	0.77
		+	0.58

Uptake of 2-aminoisobutyric acid: The uptake of this nonmetabolized and nonprotein amino acid behaved almost identically with that of 6-deoxy-D-glucose in leaves, the accumulation ratios after 2 h being 1.50 in untreated leaves and 1.39 in once-sprayed leaves, there being virtually no effect of adding cytokinin to the experimental vessel. However, with the root tissue the uptake was generally much slower, the accumulation ratio being 0.50 in untreated plant roots and 0.25 in treated plant roots. Again, the acute effect of the cytokinin was virtually undetectable. (Measurements were not done with the old plants.)

In general, the uptake of both nonelectrolytes was a slow process which, however, proceeded or would have proceeded (given enough time) against a concentration gradient within the tissue cells. Obviously, the process is one of active transport

(presumably, by analogy with other plants, a proton symport), rather than diffusion into the apoplast where no concentration gradients are likely to be formed in nonintegral tissue pieces.

The cytokinin clearly slows down the transmembrane movement of both the tested monosaccharide and the amino acid which, however, may not be in any relationship with the accumulation of sucrose in the root.

Proton adenosinetriphosphatase: Following earlier experiments with barley roots (Weisenseel *et al.* 1979, Glass and Siddiqi 1981, Behl and Raschke 1987), vine roots (Mengel and Mallisiovas 1982) and maize roots (Kotyk *et al.* 1991), root slices of sugar beet were immersed in a solution of 50 mM KCl and 50 μM $CaSO_4$ and changes of pH were followed (Fig. 2). The figure shows both a long-term effect and an acute effect of the cytokinin in stimulating the activity of the proton ATPase, very much in analogy with fusicoccin (Marrè 1979, Kotyk *et al.* 1991). The effect, however, was readily observed only in young plants examined in mid-July. Plants examined in late September showed no effect of either field spraying with the cytokinin or of its addition to the pH-electrode vessel. There was, however, a slight enhancement of the acidification by 50 μM fusicoccin.

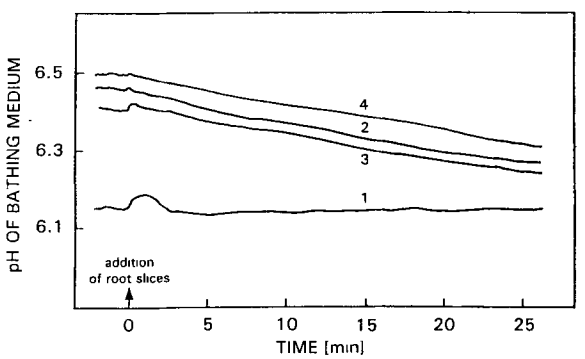

Fig. 2. pH traces of a suspension of sugar-beet root slices from untreated plants (*curve 1*), from untreated plants with the addition of 50 μM (*m*OH)[9R]BAP (*curve 2*), from (*m*OH)[9R]BAP-treated plants (*curve 3*) and from such plants with the addition of 50 μM (*m*OH)[9R]BAP (*curve 4*).

The reason for the lack of cytokinin effect on ATPase activity in older plants is difficult to assess but may be due to a virtual absence of the enzyme from the root tissue during later stages of sugar-beet growth.

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