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Seasonal changes in sugar beet photosynthesis as affected by exogenous cytokinin N^6-(*m*-hydroxybenzyl)adenosine

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Abstract

Foliar sprays with N^6-(*m*-hydroxybenzyl)adenosine, (*m*OH)-[9R]BAP, one of the synthetic cytokinins, were applied to field-grown sugar beet twice during the vegetation period: before full canopy closing (*I*), 6 weeks before harvest (*II*) or both. Application of (mOH)[9R]BAP retained high cytokinin content that usually declines prior to harvest. The total content of isoprenoid cytokinins at harvest was 2.6-fold higher in (*m*OH)[9R]BAP-treated plants as compared to the controls. Treatment *I* had no significant effect on contents of chlorophylls (Chl) *a* and *b* and carotenoids, nor on the rates of net photosynthesis (P_N) or photorespiration (R_L), or on CO_2 compensation concentration (Γ) measured during the whole vegetation period on detached leaves under optimum environmental conditions. Increased values in P_N, R_L and Chl *a* and *b* contents were found in variants *II* and *I+II* linked with a delay in leaf senescence before harvest. Transpiration rate and stomatal conductances of adaxial and abaxial epidermes were not significantly affected by any treatment.

Additional key words: *Beta vulgaris*, CO_2 exchange, photorespiration, respiration, stomatal resistance, synthetic cytokinin, transpiration.

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Abbreviations: c_a - ambient CO_2 concentration; Car - carotenoids; Chl - chlorophyll; d.m. - dry mass; E - transpiration rate; f.m. - fresh mass; g_{ab}, g_{ad} - stomatal conductance of abaxial and adaxial epidermes; iP - isopentenyladenine; P_N - net photosynthetic rate; R_D - dark respiration rate; R_L - photorespiration rate; Z - zeatin; Γ - CO_2 compensation concentration; [9R]ip - isopentenyladenosine; [9R]-Z - zeatin riboside.

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Introduction

Exogenous application of synthetic cytokinins affects the accumulation of natural endogenous cytokinins, and many processes closely related to yield formation (delay of senescence, resistance of plants to various forms of stress, respiration, source-sink relationship, *etc.*) (*e.g.*, Kamínek 1992). Cytokinins also affect photosynthetic processes both directly (*e.g.*, chlorophyll and photosynthetic protein synthesis and degradation, chloroplast composition and ultrastructure, electron transport, opening of stomata) and indirectly, by changes in growth, morphology, and anatomy (for review see Synková *et al.* 1996; *cf.* also Luštinec *et al.* 1984, Chernyadev *et al.* 1986, Kuraishi *et al.* 1992, Musgrave 1994, Wilhelmová and Kutík 1995).

In the context of a project targeted at increasing sugar yield and quality in sugar beet, the effect of several cytokinins, among them N^6-(*m*-hydroxybenzyl)adenosine, (*m*OH)[9R]BAP (Kamínek *et al.* 1987), was followed on a variety of processes involved in sugar formation (membrane transport, photosynthesis, respiration, growth rate, biomass accumulation and distribution, sugar content, technological quality, *etc.* - Pulkrábek 1990, 1995, Zahradníček *et al.* 1995, Kotyk *et al.* 1996). The present report concerns one section of the project, viz. seasonal changes in natural cytokinins and selected aspects of CO_2 exchange and water relations which may play an important role in dry matter and saccharide formation.

Materials and methods

Plants and treatments: Sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll, cv. Edda) was grown in 1994-1995 under the usual agrotechnics in a field at the Experiment Station of the Faculty of Agriculture of the Czech Agricultural University in Červený Újezd, Central Bohemia (plots 15 m^2, 4 replications).

The synthetic cytokinin N^6-(*m*-hydroxybenzyl)adenosine ((*m*OH)[9R]BAP) was obtained from Dr. T. Vaněk, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague. The (*m*OH)[9R]BAP, which exhibits 10 times higher biological activity than the most active natural cytokinin *trans*-zeatin (Kamínek *et al.* 1987), was applied as a foliar spray (3 μM in 0.03 % ethanol, together with the tenside *Cytovet* at 80 mm^3 dm^{-3}, 20 cm^3 m^{-2}) to plants before full canopy closing (variant *I*), 6 weeks before harvest (variant *II*), and at both occasions (variant *I+II*). Control plants were sprayed with the same solution without the cytokinin.

During the vegetation period of 1994, whole plants were sampled at 2-week intervals and the parameters of CO_2 exchange and water relations were measured on the 4^{th} and 5^{th} leaves from the top in the laboratory under conditions optimal for each parameter. A similar protocol was used in the vegetation period of 1995, but the plants were only sampled twice after the first (*m*OH)[9R]BAP application, and then three times after the second application. The data were statistically treated by Student's *t*-test.

Chlorophyll and carotenoid contents were determined in 80 % acetone extracts of leaves with spectrophotometer *PU-8740* (*Philips Scientific*, Cambridge, UK) according to Arnon (1949) and Lichtenthaler (1987), respectively.

Extraction and determination of cytokinins: Cytokinins were extracted and purified by a modified method of Morris *et al.* (1982). Tissues frozen in liquid nitrogen were ground to a powder and extracted with 80 % (v/v) methanol [0.1 g(f.m.) cm^{-3}]. The extract was clarified by centrifugation (10 000 *g* for 15 min) and the lipids were removed by passing the extract through a reverse-phase column (*Sep-Pak Si-C_{18}m, Waters*). The methanol was evaporated under vacuum and the cytokinins were diluted in 0.1 M ammonium hydrogen carbonate (pH 7.5). The solution was applied to DEAE-*Sephadex* columns (2 cm^3), the cytokinins were retained on the attached *Sep-Pak* cartridge column (1 cm^3, *Waters*) and eluted with 5 cm^3 of methanol. Cytokinins were fractionated by HPLC on reverse-phase *Si-C_{18}* columns (RP - 18.5 μm *LiChrospher 100m, Merck*) using a gradient of acetonitrile in 40 mM triethylamine acetate, pH 3.35. Collected fractions were assayed for cytokinins by ELISA using polyclonal antibodies specific for iP and Z. Tritiated Z and iP were added at the extraction step as recovery standards.

CO_2 exchange rates were measured on detached leaf segments (sample area of about 70 cm^2). Net photosynthetic rate (P_N) was determined as CO_2 flux in a closed gas exchange system with an infrared gas analyser *Infralyt IV* (*Junkalor*, Dessau, Germany) in a CO_2 concentration range from 20 to 1200 mg m^{-3}, leaf temperature 25 °C, air humidity of 65-75 %, and a near-saturating irradiance (400-700 nm) of 860 μmol m^{-2} s^{-1} (Čatský and Tichá 1975, Kaše and Čatský 1983). Respiration rate (R_D) was measured in darkness in the same system. Photorespiration rate (R_L) and CO_2 compensation concentration (Γ) were calculated from CO_2 dependence of P_N.

Stomatal conductances of abaxial and adaxial epidermes (g_{ab}, g_{ad}) were measured by a diffusion porometer *Delta-T* (type *Mk3*, Kingston upon Thames, UK) at a temperature of 25 °C, irradiance of 860 μmol m^{-2} s^{-1}, and air humidity 50 %.

Transpiration rates (E) were determined from water loss curves measured gravimetrically on leaves originally fully turgid. Irradiance was 860 μmol m^{-2} s^{-1}, temperature 25 °C, and air humidity 35 %. E was calculated from the slope of the water loss curve (as the first derivative) in the first minutes after cutting, as it is supposed that during this time the rate of water loss corresponds to the E *in situ* under the same conditions.

Irradiance and **air humidity** were measured with a *LI 185B* radiometer with quantum sensor (*Li-Cor*, Lincoln, USA) and with a *JUMO Humitherm TDAc-70* (*M.K. Juchheim*, Fulda, Germany), respectively.

Dry mass was determined in samples oven-dried at 90 °C to constant mass.

Results

Following treatment with (*m*OH)[9R]BAP, a significant increase in fresh mass of tap root and hence of total biomass was found at harvest in all the variants and in both the years studied (Table 1). The technological quality of the tap root was not affected. In 1994, where growth was retarded due to a drought period in July, a high increase in tap root yield (15 %) and also increase in white sugar yield (14 %) were found.

Table 1. Selected growth (fresh mass) and technological parameters of sugar beet at harvest 1994. Asterisks denote at least 5 % significance against respective controls.

Cytokinin treatment	Leaves [kg m^{-2}]	Tap root [kg m^{-2}]	Total biomass [kg m^{-2}]	Root/shoot ratio	Sugar content [%]	White sugar yield [kg m^{-2}]
Control	33.72	47.61	83.33	1.41	16.37	0.649
I	33.65	49.26*	82.91	1.46	16.40	0.670*
II	34.10	49.97*	84.07	1.47	16.47	0.682*
I+II	34.10	51.26*	85.36*	1.50	16.47	0.694*

Endogenous cytokinins: The concentrations of endogenous cytokinins were determined in the 4th and 5th leaves from the top of sugar beet plants sprayed with (*m*OH)[9R]BAP 6 weeks before the harvest (variant *II*) and in the controls. The samples for analysis were collected one week before harvest (13 September) and at harvest (20 September). The main cytokinins present in sugar beet leaves were iP and [9R]iP while zeatin-type cytokinins were found in very low amounts. The total cytokinin content in controls decreased to less than 30 % during the last week before the harvest. The iP was responsible for this rapid decline. Application of (mOH)[9R]BAP retained high levels of all cytokinins but iP. The total content of isoprenoid cytokinins at the harvest was 2.6-fold higher in (*m*OH)[9R]BAP-treated plants than the controls (Table 2).

Table 2. Content of cytokinins in young leaves of sugar beet plants after single spray application of cytokinin (*m*OH)[9R]BAP 6 weeks before the harvest (variant *II*).

Period before harvest [d]	(*m*OH)[9R]BAP [μM]	Cytokinin content [μmol kg^{-1}(f.m.)]				
		iP	[9R]iP	Z	[9R]Z	total
7	0	13.10	1.93	0.10	0.02	15.15
7	3	10.39	10.33	0.04	0.06	20.82
0	0	1.63	2.75	0.11	0.01	4.50
0	3	0.87	10.65	0.15	0.01	11.68

Seasonal changes of all photosynthetic parameters depended mainly on plant age and on weather preceding the sampling (*e.g.*, drought in July and at the beginning of August 1994, and low temperatures before the last sampling at the beginning of

October). However, the response to (*m*OH)[9R]BAP treatments of all the parameters measured was similar in the 1994 and 1995 vegetation periods.

Chloroplast pigments: Changes in contents of photosynthetic pigments after (*m*OH)[9R]BAP treatments were not significant in the year 1994 and in spring 1995. An increase in chlorophyll (Chl) *a+b* content per unit dry mass and a decrease in carotenoid (Car) contents per unit leaf area were found in (*m*OH)[9R]BAP treated plants in autumn 1995 (Fig. 1). The differences in Chl and Car contents expressed on other bases were just below 5 % confidence limits. Slight effects of (*m*OH)[9R]BAP were found on Chl *a/b* ratio and Chl/Car ratio.

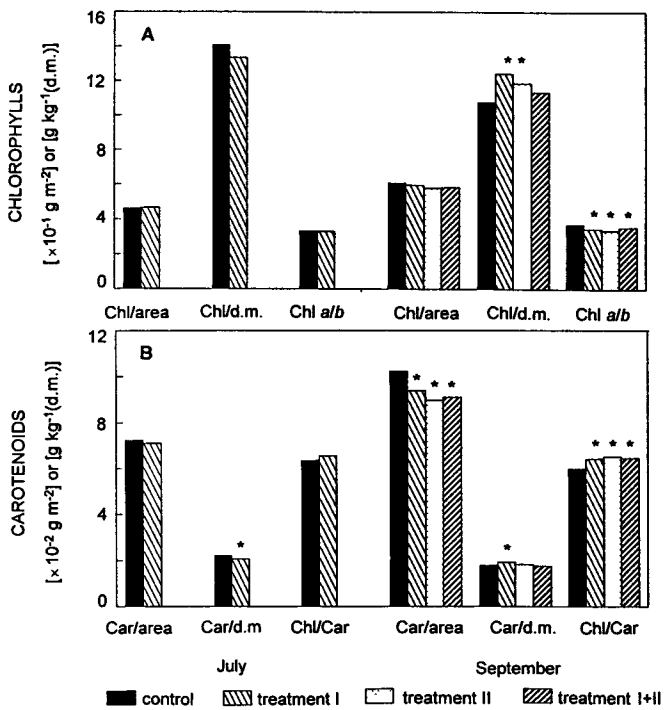

Fig. 1. The effects of (*m*OH)[9R]BAP treatments done in spring (*I*) and in autumn (*II*) on chloroplast pigment (*A* - chlorophyll, *B* - carotenoids) contents in July and September 1995: contents on area and dry mass (d.m.) bases, and Chl *a/b* and Chl/Car ratios, respectively.

CO_2 exchange rates: Typical seasonal changes in P_N and further parameters of CO_2 exchange were found in the two years of experiments. Application of (*m*OH)[9R]BAP at canopy closing (variant *I*) had no significant effect on P_N, R_L, R_D, and Γ, but a markedly higher P_N was found after the second treatment (Fig. 2). Higher P_N before harvest was associated with higher R_L. The R_L/P_N ratio varied between 0.11 and 0.19 in individual measurements; means of controls and treatments *I, II,* and *I+II* were 0.157, 0.146, 0.149 and 0.134, respectively. In all the variants,

a tendency to lower leaf R_D was found which increased before the end of vegetation period.

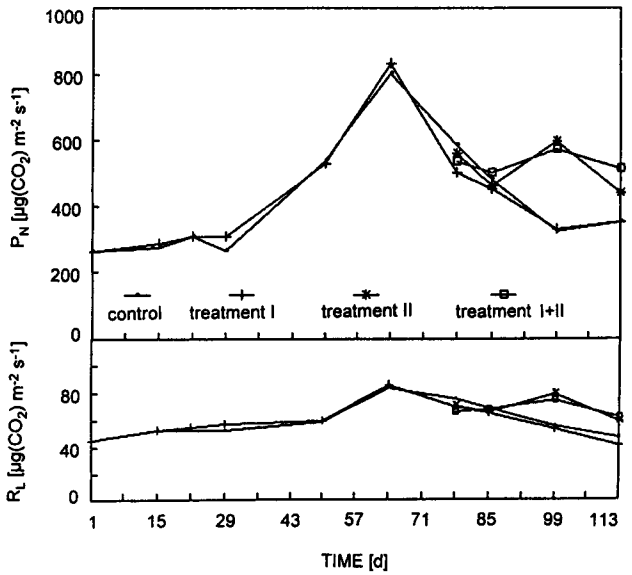

Fig. 2. The effects of (*m*OH)[9R]BAP treatments done in spring (*I*) and in autumn (*II*) on seasonal changes (1994) in the rates of net photosynthesis (P_N) and photorespiration (R_L) measured on detached sugar beet leaves under 600 mg(CO_2) m^{-3}, 25 °C and near saturating irradiance of 860 µmol m^{-2} s^{-1}. The slow increase in P_N and R_L after the first spray (*I*) with (*m*OH)[9R]BAP was associated with a 3-week period of drought.

Stomatal conductance: The effects of (*m*OH)[9R]BAP (treatments *I*, *II* and *I+II*) on g_{ab} and g_{ad} and on E were not statistically significant in either year (Fig. 3).

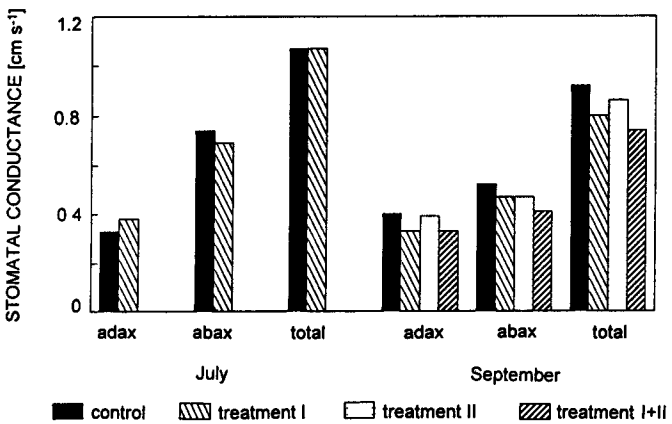

Fig. 3. Adaxial (adax), abaxial (abax) and total stomatal conductances for water vapour transfer after (mOH)[9R]BAP treatments *I*, *II* and both.

Discussion

Application of exogenous (mOH)[9R]BAP before harvest delayed senescence of young leaves as indicated by the higher content of chlorophyll content and higher P_N and R_L. This effect was associated with an increase in the content of natural cytokinins (Table 2). This corresponds to findings of other authors on the inductive effects of exogenous synthetic cytokinins on the accumulation of endogenous natural cytokinins (Vaňková *et al.* 1987, 1991). Present experiments do not allow a conclusion as to whether the effect of (mOH)[9R]BAP on the senescence of leaves is direct or mediated by endogenous cytokinins. In any case the delay of senescence is favourable for yield increase. The changes in concentrations of endogenous cytokinins might also affect the post-harvest processes in sugar beet, especially during the storage.

The seasonal changes found in photosynthesis and related processes of field-grown sugar beet agree fully with the numerous reports in the literature (*cf.* reviews in Šesták 1985, Čatský and Šesták 1996). The ontogenetic changes in photosynthesis may be markedly modified by different environmental stresses, and also by nutrient supply and growth regulators. Different environmental conditions during the years 1994 and 1995 (especially the unusual drought at the beginning of summer 1994) were the main causes of very high variability in the parameters measured, especially on stomatal conductance. Therefore, the rather slight effects of (*m*OH)[9R]BAP application on stomatal conductance in the two years and on photosynthetic pigments in the year 1994 were not statistically significant.

The most marked effect of cytokinins on photosynthetic processes is the delay of senescence, including also the retardation of age-dependent degradation of the photosynthetic apparatus. This phenomenon was confirmed also by our measurements. The application of (*m*OH)[9R]BAP in the period of intensive growth, characterised also by rapid increases in photosynthetic traits, was not significantly effective in our experiments. Markedly higher P_N found after the treatments *II* and *I+II* was associated with higher Chl content while g_{ab} and g_{ad} were not significantly affected. This confirms the above report that the effect of (*m*OH)[9R]BAP on P_N and Chl content was due to the retardation of leaf senescence, frequently found after application of exogenous cytokinins or in connection with increased levels of endogenous cytokinins (*e.g.*, Soejima 1992, Synková *et al.* 1996).

The seasonal course of R_L follows generally the seasonal course of P_N. However, the R_L values obtained by the method used in this study may be affected by additional flux of respiratory CO_2 from achlorophyllous tissues in light. This may increase the R_L values found after the treatment *II*.

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