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Dedicated to Prof. dr. MERCEDES WRISCHER
on the occasion of her 70th birthday.

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THE INTERACTION OF JASMONIC ACID, SUCROSE AND LIGHT IS REFLECTED IN PHOTOSYNTHETIC PIGMENT METABOLISM IN POTATOES GROWN *IN VITRO*

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We previously demonstrated that sucrose and jasmonic acid (JA) interact duringin photosynthetic in the regulation of development and pigment metabolism of the potato (*Solanum tuberosum* L cv. Sante) grown *in vitro*. In this study, the research was extended to the effect of light irradiance and quality on sucrose and JA mediated changes. Potato plantlets grown from stem node culture were grown on a medium supplemented with 30 mM or 90 mM sucrose and 0.1, 1, or 10 μM JA at 55–70 μmol m^{-2} s^{-1} (Osram Cool White lights) or at 80–100 μmol m^{-2} s^{-1} (Osram Fluora lights). Higher amounts of chlorophyll were detected in plants grown at lower irradiance while there was no significant difference in carotenoid levels. Sucrose and JA both enhanced root growth and inhibited photosynthetic pigment accumulation. Most effective was 1 μM JA at higher sucrose concentrations. In these growth conditions the plantlets were hyperhydric and had the most developed root system. The present study demonstrates that the growth of plants *in vitro* and the photosynthetic pigment metabolism are highly dependent on the interaction of a number of environmental factors such as sucrose and plant growth regulators in the medium, and light irradiance and quality. This result supports our previous findings that the reduction of photosynthetic pigment accumulation in cv. Sante potato plantlets induced by JA could be mainly the consequence of enhanced import of sucrose from the medium.

Key words: *Solanum tuberosum;* tissue culture; jasmonic acid; sucrose uptake; irradiation, photosynthetic pigments.

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Introduction

Plantlets grown *in vitro* exhibit both a modified structure and modified physiological functions of the photosynthetic apparatus as a result of the particular conditions: low irradiance, low CO_2, and a high sucrose level. The regulation of chlorophyll synthesis by saccharides and light irradiation in tissue cultures has already been reported (EDELMAN and HANSON 1971, SCHAFER and SCHMIDT 1991, POSPIŠILOVA et al. 1992, TICHA et al. 1998).

Jasmonates are endogenous regulatory substances, ubiquitous in the plant kingdom. Putative roles of jasmonic acid (JA) and other jasmonates range from involvement in response to wounding and pathogens, to the control of plant development (reviewed in SEMBDNER and PARTHIER 1993). Whereas high concentrations of JA often lead to growth inhibition and senescence, lower concentrations result in stimulatory effects. Many data are available on the influence of jasmonates on chlorophyll and fewer on carotenoid content. In most cases their decrease was observed in relation to growth inhibition and senescence (UEDA et al. 1981, MIERSCH et al. 1986, WEIDHASE et al. 1987, HERRMANN et al. 1992).

In our previous investigations we demonstrated that JA can beneficially influence the growth of plants in tissue culture as shown by enhanced axillary shoots and lateral root growth of the potato, bean, and grapevine, enhanced rhizoid formation in fern gametophytes, and stimulated shoot and bud development of isolated basal plates of garlic (RAVNIKAR et al. 1992, RAVNIKAR et al. 1995, CAMLOH et al. 1996). However, in the potato cultivar Sante, JA in interaction with sucrose can induce hyperhydricity of shoots (KOVAČ and RAVNIKAR 1994). According to our previous results sucrose and JA interact in the regulation of development and photosynthetic pigment metabolism in the potato cv. Sante grown *in vitro* (KOVAČ and RAVNIKAR 1998). In this study the research was extended to take in the effect of light irradiance and quality on sucrose- and JA- mediated changes.

Material and methods

Plant material

Potato (*Solanum tuberosum* L., cv. Sante) stem node cultures were grown on Murashige and Skoog (MS) medium (MURASHIGE and SKOOG 1962) supplemented with 30 or 90 mM sucrose and 0.1, 1, or 10 μM (±) rac-jasmonic acid (Apex Organix, UK). Medium without jasmonic acid (JA) was used as the control. Cultures were kept at 20±2 °C, with a photoperiod of 16 h at 55–70 μmol $m^{-2}s^{-1}$ (Osram Cool White lights), the lower irradiance, or 80–100 μmol m^{-2} s^{-1} (Osram Fluora lights), the higher irradiance. After four weeks of cultivation, the leaves of axillary shoots were cut from the plantlets and immediately frozen.

Pigment analysis

The photosynthetic pigments were extracted with cold acetone (10 mL/0.25–1g fresh weight) and analysed by reversed phase HPLC as described by KOVAČ and RAVNIKAR (1998). The calculated pigment contents are the means of the measurement of three extracts obtained from two experiments. Student's t-test was used to assess the significance of differences in values measured

between plants grown on control medium and on media supplemented with JA. The symbols used in the figures and tables are: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Results

In Figure 1 the effect of sucrose and light irradiation on photosynthetic pigment metabolism is shown. The inhibition of photosynthetic pigment accumulation by sucrose was somewhat less pronounced at the lower than at the higher irradiance. Application of JA also resulted in a decrease of both chlorophyll and carotenoid types of pigments; only in the plantlets grown with the lower amount of sucrose and 0.1 μM JA were pigment levels unaffected (Tabs. 1 and 2). Plantlets grown with the higher amount of sucrose contained particularly low amounts of pigment, in particular if grown with 1 μM JA. At these growth conditions approximately 50 % lower amounts of chlorophylls and carotenoids were detected. However, while overall pigment accumulation was always inhibited by JA, the amounts of the xanthophyll cycle pigments antheraxanthin and zeaxanthin tended to increase in the plantlets grown in the presence of the higher concentration of sucrose and 0.1 μM JA or 1 μM JA (Tab. 2). Figure 2 illustrates the influence of growth conditions on the pool of xanthophyll cycle pigments. JA treatment resulted in enhanced de-epoxidation only in the plantlets grown on media with a higher concentration of sucrose supplemented with 0.1 or 1 μM JA, as indicated by a decrease in violaxanthin content and an increase in antheraxanthin and zeaxanthin. Treatment with JA did not significantly change the chlorophyll a/b ratio, although the lowest ratio was found in the plantlets with enhanced de-epoxidation.

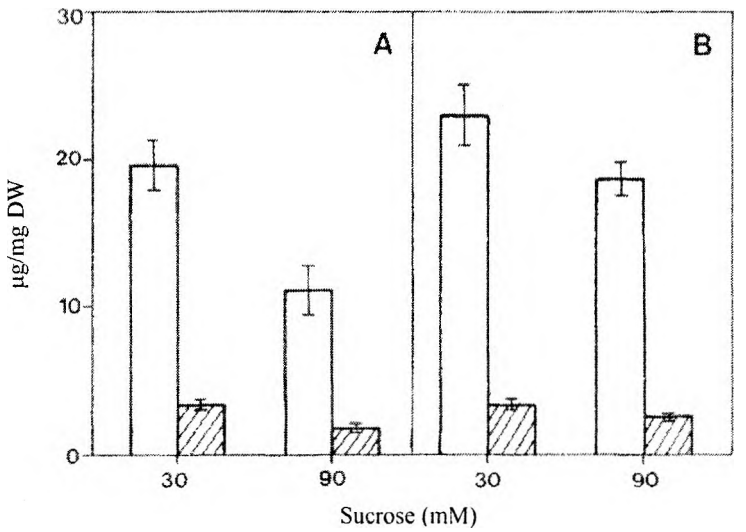

Fig. 1. Chlorophyll (□) and carotenoid (▨) contents in the leaves of potato plantlets grown at higher (A) or lower irradiance (B) on media supplemented with 30 mM or 90 mM sucrose. The calculated pigment contents are the means of the measurement of three extracts obtained from two experiments (means±SE).

Tab. 1. Levels of photosynthetic pigments in untreated and JA treated leaves of potato grown at lower light on the medium supplemented with 30 mM sucrose. The concentrations of 0.1, 1 or 10 μM JA were added to the medium. Medium without JA was used as a control. The calculated pigment contents are the means of the measurement of three extracts obtained from two experiments (mean±SE). Significance (t-test): *p<0.05, **p<0.01, ***p<0.001.

Pigment (μg/mg DW)	Controls	0.1 μM JA	% of control	1 μM JA	% of control	10 μM JA	% of control
Chlorophylls							
a	18.06 ± 1.63	19.80 ± 1.10	110	14.29 ± 2.28	79	13.84 ± 1.15	77
b	5.05 ± 0.43	5.61 ± 0.17	111	4.07 ± 0.67	81	3.96 ± 0.41	78
Total	23.11	25.41	110	18.36	79	17.80	77
Chl a/b[b]	3.6	3.5		3.5		3.5	
Carotenoids[a]							
β-carotene	0.74 ± 0.11	0.79 ± 0.09	107	0.47 ± 0.09	62	0.50 ± 0.01	68
Lutein	1.55 ± 0.17	1.62 ± 0.11	105	1.15 ± 0.18	74	1.16 ± 0.07	75
c-Neoxanthin	0.41 ± 0.06	0.41 ± 0.05	100	0.27 ± 0.06	66	0.30 ± 0.02	73
t-Violaxanthin	0.40 ± 0.04	0.44 ± 0.04	110	0.28 ± 0.05	70	0.29 ± 0.01*	73
Antheraxanthin	0.025 ± 0.008	0.02 ± 0.001	80	0.016 ± 0.002	64	0.016 ± 0.0005	64
Zeaxanthin	0.11 ± 0.005	0.12 ± 0.03	109	0.09 ± 0.02	82	0.08 ± 0.01	73
Total	3.25	3.40	105	2.28	70	2.34	72

[a]c-, 9' -cis; t-, all-trans. [b]Chl a/b, Chlorophyll a/b;

Tab. 2. Levels of photosynthetic pigments in untreated and JA treated leaves of potato grown at lower light on the medium supplemented with 90 mM sucrose. The concentrations of 0.1, 1 or 10 μM JA were added to the medium. Medium without JA was used as a control. The calculated pigment contents are the means of the measurement of three extracts obtained from two experiments (mean±SE). Significance (t-test): *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Pigment (μg/mg DW)	Controls	0.1 μM JA	% of control	1 μM JA	% of control	10 μM JA	% of control
Chlorophylls							
a	14.54 ± 0.88	9.52 ± 1.22**	65	7.35 ± 0.99***	51	9.69 ± 0.6***	67
b	4.24 ± 0.28	3.02 ± 0.30*	71	2.35 ± 0.30***	55	2.87 ± 0.22**	68
Total	18.78	12.54	67	9.70	52	12.56	67
Chl a/b[b]	3.4	3.2		3.1		3.4	
Carotenoids[a]							
β-carotene	0.51 ± 0.05	0.29 ± 0.08*	57	0.25 ± 0.04**	49	0.29 ± 0.03**	57
Lutein	1.17 ± 0.08	0.83 ± 0.12*	71	0.66 ± 0.09**	56	0.79 ± 0.05**	68
c-Neoxanthin	0.31 ± 0.02	0.22 ± 0.02*	71	0.17 ± 0.02***	55	0.18 ± 0.01***	58
t-Violaxanthin	0.36 ± 0.03	0.23 ± 0.03*	64	0.18 ± 0.02***	50	0.21 ± 0.01***	58
Antheraxanthin	0.019 ± 0.004	0.021 ± 0.005	111	0.040 ± 0.01	210	0.013 ± 0.002	68
Zeaxanthin	0.09 ± 0.01	0.10 ± 0.02	111	0.13 ± 0.04	144	0.05 ± 0.004**	56
Total	2.45	1.69	69	1.43	58	1.53	62

[a]c-, 9'-cis; t-, all-trans. [b]Chl a/b, Chlorophyll a/b;

The biochemical changes induced by different sucrose and JA concentrations are also reflected by changes of plantlet morphology, which were more pronounced in the plantlets grown with 90 mM sucrose and 1 μM JA, in which case hyperhydric symptoms appeared. The growth conditions also affected the root dry weight (Fig. 3). Sucrose and JA both enhanced root growth. The concentration of 1 μM JA was most effective at both sucrose concentrations.

Discussion

The results of the present study show that the effect of sucrose and JA in the media is influenced by the light conditions in the growth chambers. Plantlets grown at lower irradiation on the medium supplemented with less sucrose contained the highest level of photosynthetic pigments. Diminishing one of the sources of energy, that is: either the sucrose in the medium or the irradiance, led to an increase of photosynthetic pigments. The effect of irradiance on chlorophylls was expected. In many species one of the key factors that permit plants to tolerate low irradiance is the ability efficiently to harvest available photosynthetically active radiation. Regulation by light quantity and light quality is highly complex, and changes in both parameters occur simultaneously in nature (LIU et al. 1993). Also, numerous physiological and biochemical studies with sugar feeding suggest a feedback inhibition of photosynthesis (EDELMAN and HANSON 1971, KRAPP et al. 1991, SCHÄFER et al. 1992, ECKERMANN and BAUMANN 1995).

The effect of different concentrations of JA and sucrose on plant morphology and individual photosynthetic pigments was studied in the plants grown at lower irradiation (55–70 μmol $m^{-2}s^{-1}$). Except at low concentrations of JA (0.1 μM) and sucrose (30 mM), JA induced a proliferation of the root system and a decrease in carotenoids and chlorophylls. A similar but more pronounced effect of JA on plant morphology and photosynthetic pigments metabolism was found in our previous study when the plants were grown at 80–100 μmol $m^{-2}s^{-1}$ (KOVAČ and RAVNIKAR 1998). The effect of JA is dependent on sucrose level in the media. The most highly expressed changes were observed in the plantlets grown with 90 mM sucrose and 1 μM JA. In these growth conditions an increase in the level of the carotenoids antheraxanthin and zeaxanthin, the result of enhanced de-epoxidation of violaxanthin, was observed. These compounds are included in the xanthophyll cycle, or violaxanthin cycle, which is involved in the dissipation of excess excitation energy (DEMMING-ADAMS and ADAMS 1996). This result thus suggests that even though the plantlets were grown at very low irradiance, a high sucrose level and the 1 μM JA present in the medium might result in conditions such that more light was being absorbed than could be utilised in photosynthesis. At these concentrations of sugar and JA the plantlets also had the most developed root system, which might be the reason for the over-accumulation of sucrose. We previously demonstrated that exogenously applied 1 μM JA significantly stimulated radioactive sucrose uptake into potato plantlets cv. Sante grown at 80–100 μmol $m^{-2}s^{-1}$ (KOVAČ and RAVNIKAR 1998).

In conclusion, the results of this study support our previous findings (KOVAČ and RAVNIKAR 1998) that the reduction of photosynthetic pigment accumulation and xanthophyll cycle pigment de-epoxidation in cv. Sante potato plants induced

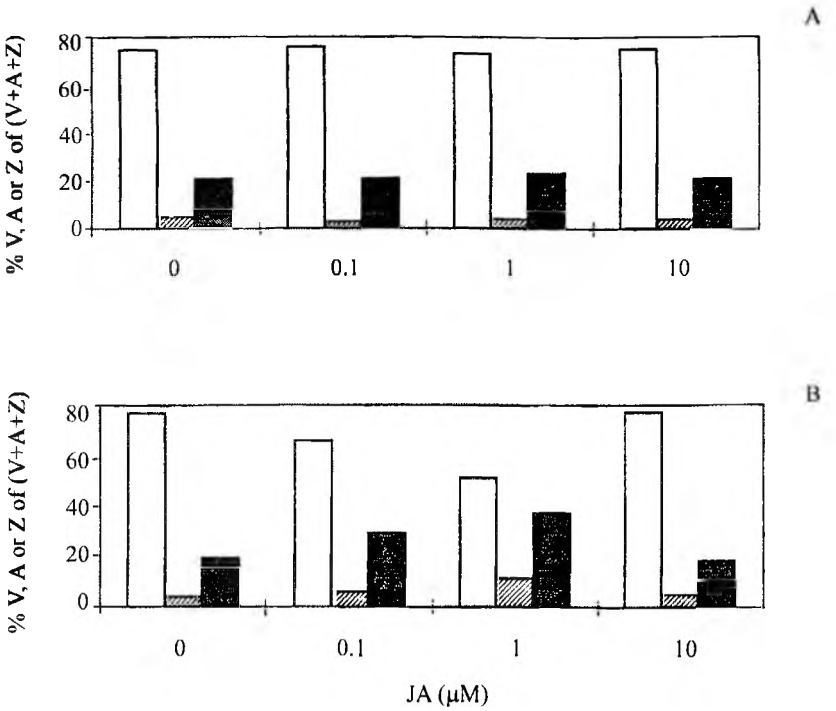

Fig. 2. The effect of 0.1, 1, and 10 μM jasmonic acid (JA) on violaxanthin (V, □), antheraxanthin (A, ▨) and zeaxanthin (Z, ■) expressed in % of (V+A+Z) in the leaves of potato plantlets grown at lower irradiance on media supplemented with 30 mM (A) or 90 mM (B) sucrose. Medium without JA was used as the control medium (0).

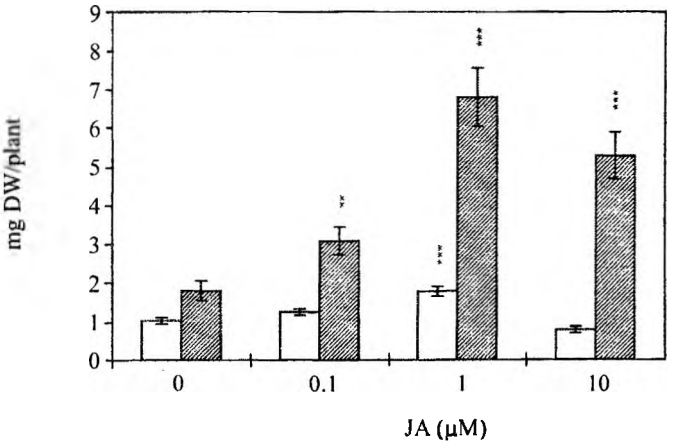

Fig. 3. The effect of 0.1, 1, and 10 μM jasmonic acid (JA) on adventitious roots' dry weight of potato plantlets grown at lower irradiance on the media supplemented with 30 mM (□) or 90 mM (▨) sucrose. Medium without JA was used as the control medium (0). Each value represents the mean of the measurements of twenty-two plants (means±SE). Significance (t-test): * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

by JA might be mainly the consequence of enhanced import of sucrose from the medium. The light irradiance affects the JA-induced changes, which are similar at low and high irradiance, but less pronounced, at low irradiance. The present study also demonstrated that the growth of plants *in vitro* is highly dependent on the interaction of a number of environmental factors such as sucrose and plant growth regulators in the medium, and light irradiance and quality.

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