Effect of Sucrose Level on the Photosynthetic Ability of In Vitro Cultivated Apple Rootstock MM 106

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Abstract

The aim of the present research was to observe the effect of sucrose level in the nutrient media on the photosynthetic ability of in vitro cultivated apple rootstock MM 106. The plantlets in an elongation stage (hormone-free medium) were grown at different sucrose concentrations (0, 1, 2 and 4% w/v) in the medium and in vessels with two types of closures – tight closure (T – gas exchange rate of 0.013 volumes/hour) and gas-permeable closure (G – gas exchange rate of 3 volumes/hour). The net photosynthetic rate (light and CO_2 curves), chlorophyll fluorescence and chlorophyll content were observed. There was a positive correlation between sucrose concentration and accumulation of dry matter and light-harvesting pigments. However, the increased sucrose concentration leads to a decrease in the net photosynthetic rate (Pn) of the plantlets. Plantlets cultivated with gas-permeable closure without sucrose showed the highest value of the Genty-parameter and ETR. The highest increase of the leaf area was achieved in plantlets cultivated on medium with 1% sucrose and gas-permeable closure. Results from this study showed that in vitro cultivated apple plantlets could develop a positive carbon balance at suitable parameters of environmental factors (mainly light intensity, CO_2 concentration and sucrose in the nutrient medium).

INTRODUCTION

The conventional in vitro technique for plant cultivation is based on the concept that microplants do not undergo photosynthesis or, in case they do, it proves to be insufficient to meet the structural and energetic requirements of their growth (Grout and Ashton, 1978). That is why they are provided with an external carbohydrate source, most frequently sucrose, which is constantly included in the nutritional medium in comparatively high concentration – 2-5%. The typical in vitro conditions – high level of humidity, low intensity of light and the exogenous carbohydrates modify the anatomy of the in vitro-formed leaves. They are characterized by not very well differentiated mesophyll, large intracellular spaces, impaired development of the cuticle, the epicuticular waxes as well as impaired structure and functioning of the stomata (Van Huylenbroeck and Debergh, 1996; Pospisilova et al., 1999).

Numerous surveys have shown that in vitro plants can potentially photosynthesize. However, their photosynthesis is being limited by the specific conditions in the cultural vessels (Desjardins, 1995; Kozai et al., 1997; Afreen et al., 2002).

The aim of the present research was to observe the effect of sucrose level in the nutrient media on the photosynthetic ability of the in vitro cultivated apple rootstock MM 106.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The study was carried out with the apple rootstock MM 106. The in vitro culture was maintained by transferring it every 3 weeks onto a basic MS (Murashige and Skoog, 1962) nutritional medium enriched with 4.44 µM 6-benzylaminopurine (BAP), 0.057 µM indole-3acetic acid (IAA), 30 g/L sucrose and 7.0 g/L agar (Fluka), pH 5,6 (before

autoclaving).

The photosynthetic capacity of the in vitro plants was studied at the end of the stage of elongation – 14 days on a MS hormone-free medium with 4 different sucrose concentrations (0, 1, 2 and 4%). 30 explants were inoculated in a glass jar (volume 600 ml) with 100 ml of nutritional medium. Two types of closures of the cultural vessels were used – tight closure (T) ensuring a gas exchange rate of 0.013 volumes per hour as well as gas-permeable closure (G) ensuring a gas exchange rate of 3 volumes per hour (Nacheva and Ivanova, 1998). Cultures were kept in a growth chamber at 22±2°C and under photoperiod of 16h light (40 μmol $m^{-2}s^{-1}$ PPFD – fluorescent lamps OSRAM 40 W) and 8h dark period. The leaf area and the dry matter of the leaves, stems, and total plantlets were measured. The plant material was dried at 80°C until constant values.

Chlorophyll Content

The chlorophyll content was determined spectrophotomethrically in 80% acetone extract. The amount of the leaf pigments was calculated according to the Lichtenthaler and Wellburn (1983).

Chlorophyll Fluorescence Analysis

The chlorophyll fluorescence analysis was completed with a chlorophyll fluorometer MINI-PAM (Walz, Germany) on the first fully developed leaves. Minimal fluorescence (Fo) was measured in 45 min dark-adapted leaves using weak modulated light of <0.15 μmol $m^{-2}s^{-1}$ and maximal fluorescence (Fm) was measured after 0.8 s saturating white light pulse (8000 μmol $m^{-2}s^{-1}$) in the same leaves. The maximum photochemical efficiency of PS II (maximal quantum yield) was calculated as (Fm−Fo)/Fm=Fv/Fm. In light adapted leaves steady state fluorescence yield F (after a 10-minute illumination with PAR 30 μmol $m^{-2}s^{-1}$) and maximal fluorescence (Fm*) after 0.8 s saturating white light pulse (8000 μmol $m^{-2}s^{-1}$), were determined. Actual quantum yield of PSII in light-adapted leaves (Y*) was calculated by the (Y*= (Fm*-F)/Fm*) ratio (Genty et al., 1989).

The samples were illuminated at step-wise increasing PPFD (30 to 700 μmol m^{-2} s^{-1}) with two-minute illumination periods at each and F and Fm* were registered (Schreiber et al., 1994). The relative electron transport rate (ETR) was calculated according to the equation: ETR = c × Y* × PAR, with c being a proportionality factor and Y* corresponding to the effective quantum yield.

Photosynthetic Response Curves

The gas exchange rate of plantlets was analyzed by a portable infrared gas analyzer LCA -4 (ADC, UK). The light and CO_2-curves were determined at 4 light intensities (approx. 0, 40, 120 and 250 μmol $m^{-2}s^{-1}$ PPFD) and 4 CO_2-concentrations (approx. 100, 350, 700 and 1500 vpm (μmol mol^{-1})) as described previously (Nacheva et al., 2002). Before measuring the dark respiration rate (Rd), the plants were dark adapted for at least 30 min. Using the light and CO_2-curves, the compensation point "Γ", the initial slope of both the concentration curves and the light curves (dy/dx) and the maximum net photosynthesis (Pn max) were calculated.

Chlorophyll fluorescence was carried out upon plants of four variants: T2 – 2% sucrose, tightly closed vessels; T1 – 1% sucrose, tightly closed vessels; G1 – 1% sucrose, a gas-permeable cover, and G0 – without sucrose, with a gas-permeable cover. Net-photosynthesis rates (light and CO_2 concentration curves) of plants cultivated at three sucrose concentrations (0%, 1% and 2%) and two types of vessel closures were measured (T and G).

RESULTS AND DISCUSSION

In Vitro Growth and Chlorophyll Content

The sucrose concentration in the nutritional medium and the gas exchange

between the cultural vessels and the environment had a great and significant impact on the growth of in vitro apple plantlets in the stage of elongation (Table 1). When there was no sucrose in the nutritional medium and the vessels were tightly covered (T0), the total dry biomass increment of the microplants was insignificant. The presence of sucrose with the same type of cover (T1-T4) led to a significant increase in the total growth but it did not refer to its concentration – the total biomass at the end of the subcultural period was practically the same with 1, 2 or 4%.

When cultivation was carried out in vessels with a gas-permeable cover and without sucrose in the nutritional medium (G0), total dry biomass increment was reported, which was due solely to photosynthesis. That demonstrated the actual possibility for photoautothrophy of the in vitro apple plants. There was a tendency to an increase of the total biomass of the plantlets observed in the variants with a gas-permeable cover under different concentrations of sucrose (variants G1, G2 and G4), which also might represent a certain contribution of the photosynthesis through myxothrophy.

On a sugar-free nutritional medium and under tight closure of the jars (T0), the leaf growth stopped. Moreover, the leaves lost a part of the dry biomass they had had when being set (Table 1). On the contrary, again without sucrose but with a gas-permeable cover used (variant G0), the increase of dry biomass of the leaves was distinct. The sucrose concentration in the nutritional medium did not have a significant effect on the leaf growth in biomass. Variant G1 was distinguished by the greatest growth in leaf area among the plants from one cultural vessel, thus confirming the positive impact of the vessel ventilation on the development of the photosynthetic apparatus (Table 1).

When a conventional cover was used, the chlorophyll content (a+b) increased more rapidly with the increase of the sucrose concentration up to 2% (variants T1 and T2), while with 4% or 6% sucrose (data not shown) it decreased (Table 1). Chlorophyll degradation was found when there was a lack of sucrose in the nutritional medium. The chlorophyll content was higher in the plants cultivated under better gas exchange conditions in the cultivated vessels (G series) compared to the plants from the T series at the same sucrose concentration.

Chlorophyll Fluorescence Analysis

The compared variants did not differ in their maximal quantum yield – Fv/Fm ratio varied around 0.8 – the normal range for stress-free leaves (Bolhar-Nordenkampf and Oquist, 1993), (Table 2). After the light adaptation of the plantlets, the actual quantum yield (Genty's parameter – Y*) of variant T2 was lower than in variant T1. The accelerated gas exchange between the cultural vessel and the environment led to an increase of Y* under identical sucrose concentrations in the nutritional medium (comparison between T1 and G1). The variant with stimulated photoautotrophy (G0) had the highest actual quantum yield.

The dependence of ETR on the light intensity (ETR = f(PAR)) for the examined four variants can be visualized by specific saturation curves (Fig. 1). These curves represent the light-photosynthesis relationship, expressed in relative units. The light saturation of the variant T2 occurred only with light intensity of about 150 μmol $m^{-2}s^{-1}$, while with the photoautotrophic variant (G0) ETR continued to increase until the light intensity raised above 500 μmol $m^{-2}s^{-1}$. The maximum value of ETR for the last variant in the saturation area was about three times greater than the one of the variant T2 and it was comparable to the one of ex vitro plants, which had gone through the first phase of adaptation. Therefore, optimization of the external factors in the process of in vitro cultivation could increase the photosynthetic efficiency of microplants. Moreover, such an optimization could make the plant adaptation to the ex vitro conditions easier.

Photosynthetic Response Curves

The net photosynthesis (Pn) of plants from variant T2 increased with the raise of CO_2 concentration and reached saturation about 1000 vpm (Fig. 2). Pn increased markedly faster in the variant with a gas-permeable closure (G2), where the Pn values did

not reach a plateau even at 1500 vpm. In the T2 variant, the initial slope (dy/dx) of the exponential curve was almost two times smaller, which came to prove the increased carboxylase activity in plants (G2) cultivated with a better gas exchange between the vessels and the environment (Fig. 2). In the CO_2-saturation zone, when the CO_2 photo fixation was determined by the quantum yield, their maximum Pn was almost three times greater than the one of T2 (Table 3). The higher quantum yield of the G2 plantlets was evident also from the initial slope of the light-dependence curves (Fig. 2).

With all the examined PPFD values, plantlets cultivated on a sucrose-free medium showed a significantly higher Pn in comparison with the rest of the variants (Fig. 3). When the light intensity increased to about 150 μmol $m^{-2}s^{-1}$, the Pn of the microplants from the autotrophic variant (G0) raised quickly and their quantum yield (expressed by dy/dx) was two times higher than the one with sucrose (Table 3). Under PAR values, up to 100 μmol $m^{-2}s^{-1}$, the plantlets from the variants with 1% and 2% sucrose showed close values of Pn (Fig. 3). Pn of the plants grown under photoautothrophic conditions (G0) was significantly higher (Fig. 3). This was probably related to the increased carboxylase activity (the dy/dx parameter). At a saturating light intensity and saturating CO_2 concentration revealed a marked gradation in the maximum net photosynthesis (Pn max) – the highest at 0% sucrose (G0), followed by 1% sucrose variant (G1) and lower in 2% sucrose variant (G2), (Table 3). The estimated values of the initial slope of the curves (dy/dx), which represented the carboxylase activity, were almost in the same gradation.

The obtained results confirmed the hypothesis that exogenous sugars in the nutritional medium were one of the main factors related to the low Rubisco activity in the cellular and tissue cultures, and that this decreased activity might explain the low Pn intensity (Desjardins, 1995).

The net photosynthesis of plantlets from different plant species, removed from culture vessels and measured under optimal environmental conditions, was comparable to that of the respective seedlings (Pospisilova et al., 1996). The results obtained by us for the apple rootstock MM 106, as well as for the plum rootstock Dzhanka 4 (Nacheva and Ivanova, 1998, 2006), showed that at suitable parameters of the environmental factors (mainly light intensity, CO_2 concentration and sucrose in the nutrient medium), the in vitro plants could develop a positive carbon balance, i.e., they could create their organism at the expense of their own photosynthesis. The photosynthetic productivity of plantlets could be increased significantly by adequate control of the environmental factors.

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Tables

Table 1. Effect of the sucrose concentration in the nutrient media and the type of vessel closure on photosynthetic ability of the in vitro cultivated apple rootstock MM 106.

	Tightly closed vessels (gas exchange rate 0.013 volumes/hour)				Gas-permeable closure of the vessels (gas exchange rate 3 volumes/hour)			
	Sucrose in the nutrient medium							
	0% T0	1% T1	2% T2	4% T4	0% G0	1% G1	2% G2	4% G4
Dry weight (DW) increment of the plants of 1 cultural vessel (g)	0.63±0.06*b	0.93±0.09*a	1.09±0.11 a	1.08±0.09 a	0.77±0.08*b	1.19±0.12 *a	1.20±0.12 a	1.22±0.13 a
Dry/Fresh mass ratio (%) (DM=DW/FWX100)	9.3±0.6*c	14.2±0.7*b	16.9±1.1 a	15.7±0.8**a	11.6±0.8 *c	16.0±0.9*b	18.6±1.3 ab	20.7±1.1*a
Dry/Fresh mass ratio (%) of the leaves, (DM=DW/FWX100)	11.7±0.8*c	15.0±1.8*b	20.5±1.5 a	22.4±1.7*a	13.6±1.6*d	17.7±1.2*c	22.3±2.3 b	25.2±2.6*a
Dry weight (DW) increment of the leaves of 1 cultural vessel (g)	- 0.02 a	0.184 b	0.266 c	0.274 c	0.105 a	0.366 b	0.343 b	0.341b
Increment of the leaf area of one cultural vessel (cm^2)	-29.58 a	66.28 b	66.88 b	56.47 b	59.5 a	164.65 b	85.13 a	51.79 a
Chlorophyll (a+b) (mg m^{-2})	207± 9.0**c	263±15 ab	290±14 a	245±16**b	241±20**c	285±13 b	298±24 ab	321.2±3**a

*The letter symbols in the brackets stand for the presence (different letters) or the absence (identical letters) of a statistically significant difference in the comparison between the variants by their sucrose concentration (Duncan Multiple Range Test). Every two variants with the same sucrose concentration are compared on the basis of the type of vessel closure. *P<0.05; **P<0.01.

Table 2. The maximal photochemical efficiency of PS II (maximal quantum yield - Fv/Fm) and actual quantum yield of PSII in light-adapted leaves (Genty's parameter - Y*) of in vitro apple plantlets cultivated at different conditions.

	VARIANTS			
	T2	T1	G1	G0
Fv/Fm	0,813	0,798	0,791	0,791
Y*	0,652	0,676	0,682	0,718

Table 3. Values for the functional photosynthetic parameters, calculated from the curves of net photosynthetic rate versus photon flux density (Pn=f (PAR)) at saturated CO_2-concentration (1500 vpm) and net photosynthetic rate versus CO_2-concentration (Pn=f (CO_2)) at saturated photon flux density (250 μmol $m^{-2}s^{-1}$).

Variants	Light curves Pn=f(PAR)		CO_2 -curves Pn=f(CO_2)	
	Max net photos. rate A(max) *n*molCO_2 $(gDW)^{-1}s^{-1}$	Quantum yield dy/dx	Max net photos. rate (Amax) *n*molCO_2 $(gDW)^{-1}s^{-1}$	Carboxylase activity dy/dx
G0	237.2±3.1	1.715	221.8 ±2.5	0.331
G1	226.8±1.9	0.883	158.4 ±1.6	0.236
G2	163.4±1.6	0.845	131.9± 1.4	0.187
T2	56.3±0.8	0.401	46.0± 0.6	0.099

Figures

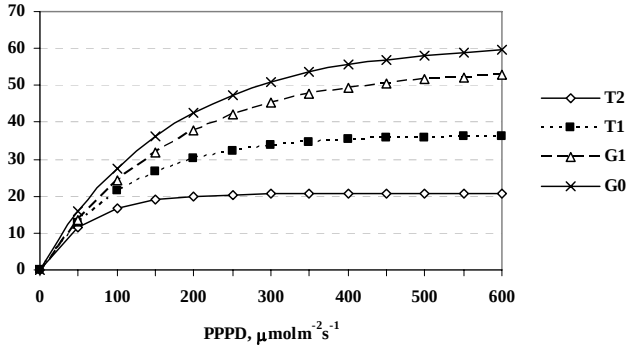

Fig. 1. Dependence of the relative electron transport rate (ETR) of in vitro apple plantlets at different conditions on the light intensity. T2 – 2% sucrose, tightly closed vessels; T1 – 1% sucrose, tightly closed vessels; G1 – 1% sucrose, gas-permeable cover, and G0 – without sucrose, with a gas-permeable cover.

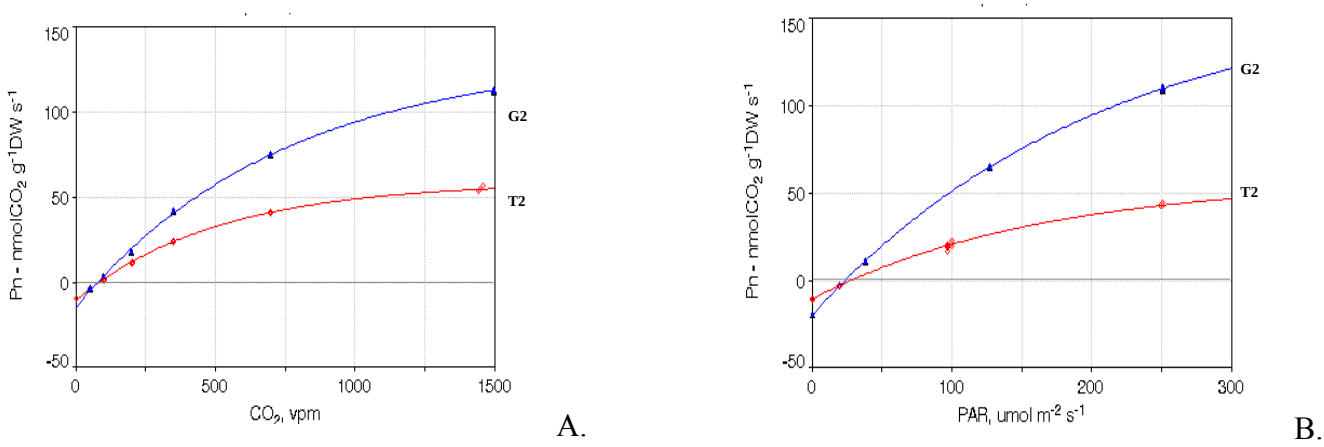

Fig. 2. CO_2-concentration curves (A) and light curves (B) of the photosynthesis of in vitro apple plants (MM 106), cultivated at 2% sucrose in the nutrient medium in the vessels with different closures - T2 - (tightly closed vessels - gas-exchange rate of 0.013 volumes/hour) and G2 - (gas- exchange rate of 3 volumes/hour).

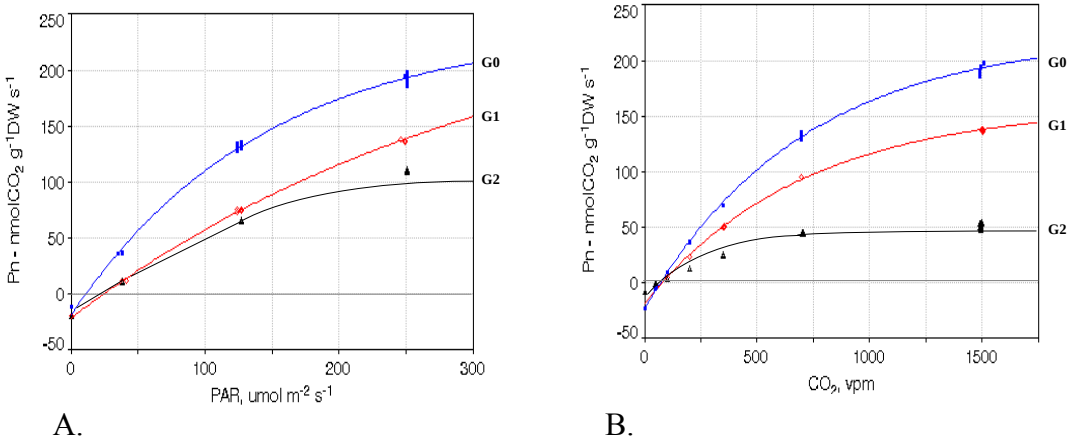

Fig. 3. Light curves (A) and CO_2-concentration curves (B) of the photosynthesis of in vitro apple plants (MM 106), cultivated in the vessels with a gas-permeable closure at different sucrose levels in the nutrient medium – G2 – 2% sucrose, G1 – 1% sucrose and G0 – without sucrose.