BIOLOGIA PLANTARUM 41 (4): 507-513, 1998

The efficiency of transfer of plants cultivated *in vitro* to *ex vitro* conditions as affected by sugar supply

Z. VORÁČKOVÁ, H. LIPAVSKÁ* and P. KONEČNÝ

Department of Plant Physiology, Faculty of Science, Charles University, Viničná 5, 128 44 Prague 2, Czech Republic

Abstract

The greatest growth of wheat and rape plants *in vitro* was reached on media with 5 or 9 % sucrose, respectively. The highest efficiency for transfer of these plants to *ex vitro* conditions was found at the same sucrose concentrations. The content of endogenous non-structural saccharides (glucose, fructose, sucrose, starch and fructans) increased with increasing sucrose concentration in the medium up to 10 %.

Additional key words: *Brassica napus*, *ex vitro* acclimation, *Triticum aestivum*.

Introduction

During *in vitro* cultivation, plantlets grow under very special conditions resulting in slow growth generally explained by their low photosynthetic ability (Langford and Wainwright 1987, Grout 1988, Kozai *et al.* 1988, Capellades *et al.* 1991, Gross *et al.* 1993). Cultivation media are therefore supplemented by saccharides. These special conditions result in the formation of plants with abnormal morphology, anatomy and physiology that can be important when the plants are to be transferred to *ex vitro* conditions (Pospíšilová *et al.* 1992). It has been found that the net photosynthetic rate of plantlets *in vitro* is not primarily restricted by the development of the photosynthetic apparatus but mainly by the low CO_2 concentration during the light period in the almost air-tight cultivation vessels (Solárová 1989, Pospíšilová *et al.* 1992).

Exogenous sugars are usually supplied to promote plant growth *in vitro*. However, in cell suspension cultures of *Chenopodium rubrum*, Schäffer *et al.* (1992) found an inhibition of photosynthesis by sugars. The accumulation of starch and soluble sugars after feeding with glucose inhibited photosynthesis in leaves of *Spinacia oleracea* (Krapp *et al.* 1991) and *Rosa* (Capellades *et al.* 1991) plantlets.

Immediately after transfer to *ex vitro* conditions the plantlet is exposed to considerable stress. The increase in photosynthetic capacity *in vitro* resulting from

Received 8 April 1998, accepted 21 July 1998.
*Corresponding author, fax: 21953306, e-mail: lipavska@prfdec.natur.cuni.cz

lowering sucrose concentration in the medium and increasing irradiance and ambient CO_2 concentration can give faster and more successful acclimatization (Desjardins *et al.* 1993, Kozai 1991, Genoud-Gourichon *et al.* 1996). On the other hand, Capellades *et al.* (1991) demonstrated that high sucrose concentrations during *in vitro* cultivation facilitate acclimatization of rose plantlets during subsequent growth *ex vitro*. Van Huylenbroeck and Debergh (1996) concluded from their experiments with *Spathiphyllum floribundum* that the primary requirement was for nutrient reserve needed to overcome transition stress, and photosynthetic activity at transplantation was of secondary importance.

In the present study the contents of nonstructural saccharides (glucose, fructose, sucrose, starch and fructans) were determined in wheat and rape plants grown *in vitro* at different sucrose concentrations. The results were correlated with *in vitro* growth and *ex vitro* plant acclimation in order to find the optimum exogenous sucrose supply (under conditions of no CO_2 enrichment) for the best transfer efficiency.

Materials and methods

Plants: Wheat (*Triticum aestivum* L. cv. Sparta) and rape (*Brassica napus* L. cv. Darmor) seeds were surface sterilized by 1.5 or 1 % NaOCl, respectively, for 20 min at 35 °C and then washed three times with sterile water. Wheat seeds were allowed to imbibe for 15 h at 25 °C and then embryos were excised. Excised wheat embryos or sterile rape seeds were placed on to the LS medium solidified by 0.7 % agar (m/v) (Linsmaier and Skoog 1965) with different sucrose concentrations (1 - 10 % for rape, 1, 3, 5, 7, 9 % for wheat). The cultures were grown at a temperature of 20 °C, 16-h photoperiod and irradiance of 150 μmol m^{-2} s^{-1} for 8 d (wheat) or 21 d (rape). After transfer to the soil, plantlets were grown in the greenhouse for 14 d. Dry mass was determined after freeze-drying.

Saccharide content determination: Freeze-dried rape leaves (roughly 20 mg) were boiled with 80 % methanol (0.5 cm^3) at 75 °C for 10 min and then the methanol was evaporated. The residue was dissolved in water in an ultrasonic bath for 10 min and filtered through membrane filters (*Whatman*, 0.45 μm pore size).

For wheat leaves the extraction method of Kubín (1994) for low-polymer sugars and fructans was modified. A frozen sample was thinly cut and homogenized in water at 70 °C for 1 min. Then oligosaccharides and low-polymer fructans were extracted in water at 70 °C, 10 min, three times. The supernatant (1000 *g*, 5 min) was frozen at -60 °C and then freeze-dried. The freeze-dried sample was dissolved in redistilled water and refined through filters.

The contents of sucrose, glucose, fructose and fructans were determined by HPLC analysis: column *Ostion LG KS 0800* Ca^{2+}, 8 × 250 mm, guard columns *Hema-Bio 1000 SB* and *Hema-Bio 1000 Q*, (*Tessek*, Prague, Czech Republic), eluent H_2O, flow rate of 0.5 cm^{-3} min^{-1}, temperature of 80 °C, refractometric detection.

Starch content was determined according to Jermin (1956). From homogenized plant material (rape) or from the sediment (after the extraction of oligosaccharides,

dried 6 h, 80 °C, wheat), starch was extracted and partially hydrolyzed by 44 % $HClO_4$ (24 h, shaking). Glucose was assayed colorimetrically after reaction with anthron.

Results and discussion

The presence of exogenous sugars in the medium affected not only the growth and development of plant *in vitro* but also the success of acclimation to *ex vitro* conditions.

The best growth of wheat plantlets *in vitro* was on a medium with 5 % sucrose (Fig. 1). These plants were also the best growing ones *ex vitro* and their increase of fresh mass during growth *ex vitro* was the highest (Fig. 1). The highest fresh mass of *in vitro* grown rape plants was on media with 8 or 9 % sucrose. After transfer to *ex vitro* condition these plants also had the highest fresh mass and the highest increase in fresh mass (Fig. 2).

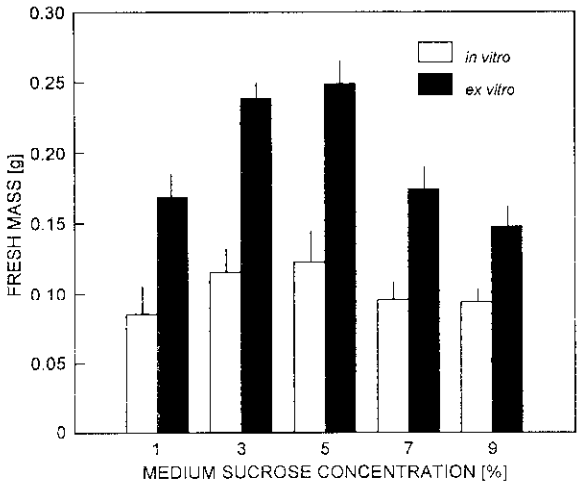

Fig. 1. Wheat plant fresh mass in dependence of the sucrose concentration in medium after 8 d of *in vitro* cultivation and a further 14 d of *ex vitro* cultivation. *Bars* indicate SD.

Large amounts of sugars accumulated in leaves can influence photosynthesis by feedback-inhibition of activity of photosynthetic enzymes (Azcón-Bieto 1983, Schäffer *et al.* 1992), but also by down-regulation of expression of genes encoding photosynthetic enzymes (Scheen 1994, Jones *et al.* 1996). It is proposed that carbohydrate repression is a mechanism for sink regulation of photosynthesis (Krap *et al.* 1993). It can therefore be expected that feeding plants with exogenous sucrose during *in vitro* cultivation might cause down-regulation of photosynthesis and adversely influence the efficiency of transfer to *ex vitro*.

Some authors, however, suggest that high amounts of reserve saccharides (sucrose, starch, fructans) are more important for plant survival than full autotrophy at the time of transfer (Capellades *et al.* 1991, Van Huylenbroeck and Debergh 1996).

In our experiments, we found that increase in exogenous sugar supply led to accumulation of nonstructural saccharides. In wheat grown on the medium with 5 %

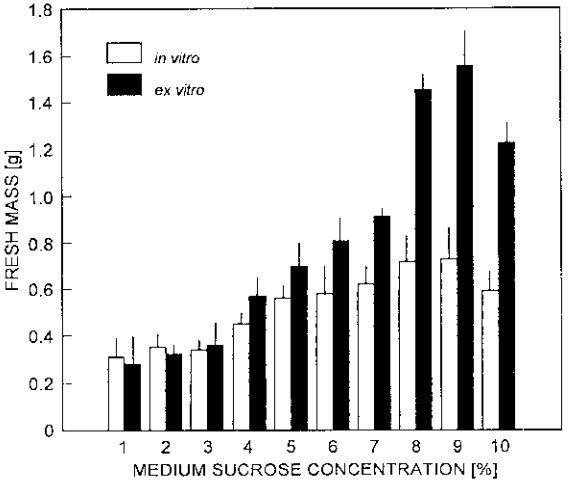

Fig. 2. Rape plant fresh mass in dependence of the sucrose concentration in medium after 21 d of *in vitro* cultivation and a further 14 d of *ex vitro* cultivation. *Bars* indicate SD.

sucrose, the contents of glucose, fructose and sucrose were nearly twice as high as in plants grown on 1 and 3 % sucrose. A further increase was found in plants grown on 7 and 9 % of sucrose (Fig. 3). Glucose was the sugar found at the highest concentration. Free hexoses such as glucose and fructose are usually much less

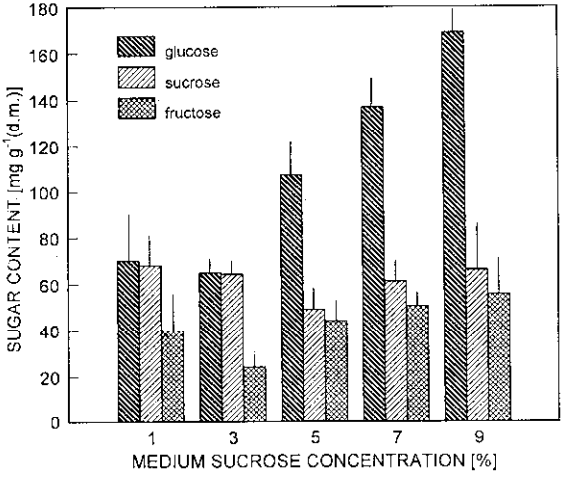

Fig. 3. Glucose, fructose and sucrose contents in wheat shoots in dependence of the sucrose concentration in medium. *Bars* indicate SD.

abundant than sucrose in photosynthetic cells although in many non-photosynthetic cells the situation is opposite (Ross 1992). In our experiments the concentrations of hexoses prevailed over that of sucrose in shoots as well as in roots (data for roots not shown). In rape the contents of sucrose and glucose were comparable but the content of fructose was lower. Nevertheless, there was an increase in sugar concentration with increasing exogenous sugar supply as well (Fig. 4).

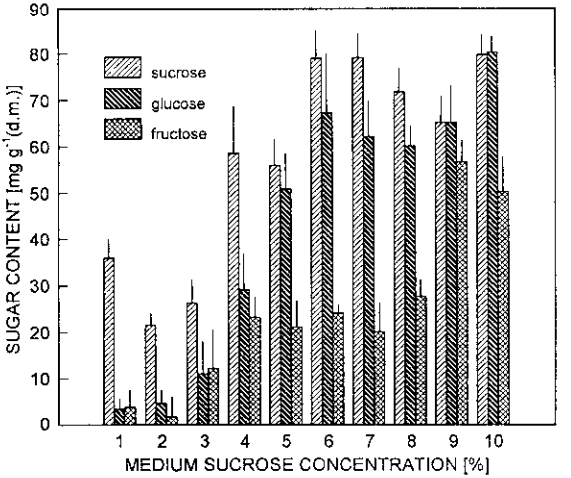

Fig. 4. Glucose, fructose and sucrose contents in rape shoots in dependence of the sucrose concentration in medium. *Bars* indicate SD.

A high amount of starch was found in shoots of plants cultivated *in vitro*. The content of starch rose in wheat (Fig. 5) and rape (Fig. 6) both in shoots and in roots

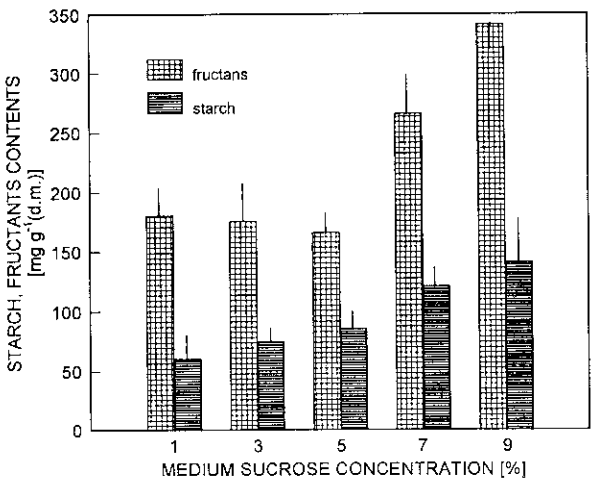

Fig. 5. The content of starch and fructans in wheat shoots in dependence of the sucrose concentration in medium. *Bars* indicate SD.

(data not shown) with rising concentration of sucrose in the medium. The content of wheat storage saccharides - fructans - increased with increasing sucrose concentration in the medium as well (Fig. 5). Capellades *et al.* (1991) found low photosynthetic rates (probably caused by starch accumulation) in plants grown *in vitro* on a high sucrose concentration, but better survival of those plants after transfer to *ex vitro* conditions. The authors suggested that storage assimilates (starch) were translocated from the chloroplast during acclimation, photosynthetic capacity was restored at the expense of starch breakdown and functional photosynthetic apparatus was rebuilt.

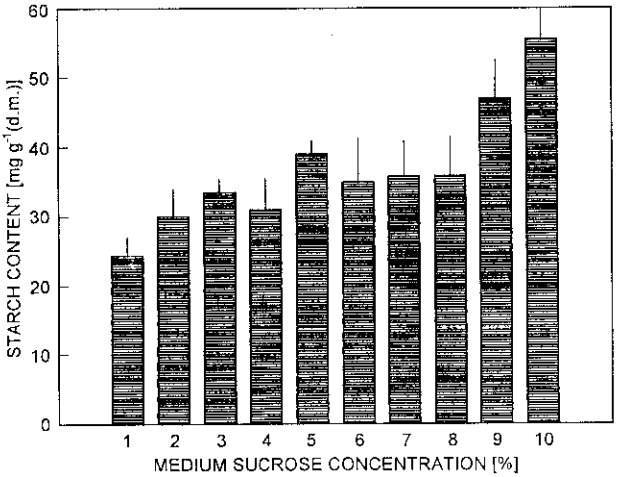

Fig. 6. The content of starch in rape shoots in the dependence of the sucrose concentration in medium. *Bars* indicate SD.

According to those results we would have expected the highest increase in plant fresh mass *ex vitro* for plants cultivated *in vitro* on the highest medium sucrose concentrations. However, the highest fresh mass after *ex vitro* cultivation was found to be for plants showing the best growth *in vitro*, that means for wheat and rape grown on 5 or 9 % sucrose, respectively. Higher concentrations of sucrose in the medium (7 and 9 % for wheat and 10 % for rape) reduced the growth rate after transplantation.

References

Azcón-Bieto, J.: Inhibition of photosynthesis by carbohydrates in wheat leaves. - Plant Physiol. **73**: 681-686, 1983.

Capellades, M., Lemeur, R., Debergh, P.: Effects of sucrose on starch accumulation and rate of photosynthesis in *Rosa* cultured *in vitro*. - Plant Cell Tissue Organ Cult. **25**: 21-26, 1991.

Desjardins, Z., Gosselin, A., Yelle, S.: Acclimatization of *ex vitro* strawberry plantlets in CO_2-enriched environment and supplementary lighting. - J. amer. Soc. hort. Sci. **112**: 846-851, 1993.

Genoud-Gourichon, C., Sallanon, H., Coudret, A.: Effect of sucrose, agar, irradiance and CO_2 concentration during the rooting phase on the acclimation of *Rosa hybrida* plantlets to *ex vitro* conditions. - Photosynthetica **32**: 263-270, 1996.

Gross, U., Gilles, F., Bender, L., Berghöfer, P., Neumann, K.H.: The influence of sucrose and an elevated CO_2 concentration on photosynthesis of photoautotrophic peanut (*Arachis hypogaea* L.) cell cultures. - Plant Cell Tissue Organ Cult. **33**: 142-150, 1993.

Grout, B.W.W.: Photosynthesis of regenerated plantlets *in vitro*, and the stresses of transplanting. - Acta Hort. **230**: 129-135, 1988.

Jermin, M.A.: A new method for determining ketohexoses in the presence of aldohexose. - Nature **177**: 38-39, 1956.

Jones, P.G., Lloyd, J.C., Raines, C.A.: Glucose feeding of intact wheat plants represses the expression of a number of Calvin cycle genes. - Plant Cell Environ. **19**: 321-236, 1996.

Kozai, K., Koyama, Z., Watanabe, I.: Multiplication of potato plantlets *in vitro* with sugar free medium under high photosynthetic photon flux. - Acta Hort. **230**: 121-127, 1988.

Kozai, T. Photoautotrophic micropropagation. - *In vitro* Cell Dev. Biol. **27**: 47-51, 1991.

Krapp, A., Quick, W.P., Stitt, M.: Ribulose-1,5-bisphosphate carboxylase-oxygenase, other Calvin-cycle enzymes and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. - Planta **186**: 58-69, 1991.

Krapp, A., Hoffmann, B., Schafer, C., Stitt, M.: Regulation of the expression of *rbc*S and other photosynthetic genes by carbohydrates: a mechanism for the sink-regulation of photosynthesis? - Plant J. **3**: 817-828, 1993.

Kubín, P.: [Changes in content of storage saccharides in wetland plants at oligotrophic and eutrophic localities.] - MSc. Thesis, Faculty of Sciences, Charles University, Prague 1994. [In Czech.]

Langford, P.J., Wainwright, H.: Effect of sucrose concentration on the photosynthetic ability of rose shoots *in vitro*. - Ann. Bot. **60**: 633-640, 1987.

Linsmaier, M.P., Skoog, F.: Organic growth factor required in tobacco tissue cultures. - Plant Physiol. **18**: 100-127, 1965.

Pospíšilová, J., Solárová, J., Čatský, J.: Photosynthetic responses to stress during *in vitro* cultivation. - Photosynthetica **26**: 3-18, 1992.

Ross, C.W.: Carbon dioxide fixation and carbohydrate synthesis. - In: Salisbury, F.B., Ross, C.W. (ed.): Plant Physiology. Pp. 244-249. Wadsworth Publishing Company, Belmont 1992.

Schäffer, C., Simper, H., Hofmann, B.: Glucose feeding results in co-ordinated changes of chlorophyll content, ribulose-1,5-bisphosphate carboxylase-oxygenase activity and photosynthetic potential in photoautotrophic suspension cultured cells of *Chenopodium rubrum*. - Plant Cell Environ. **15**: 343-358, 1992.

Schen, J.: Feedback control of gene expression. - Photosynth. Res. **39**: 427-438, 1994.

Solárová, J.: Photosynthesis of plant regenerants. Diurnal variation in CO_2 concentration in cultivation vessels resulting from plantlets photosynthetic activity. - Photosynthetica **23**: 100-107, 1989.

Van Huylenbroeck, J.M., Debergh, P.C.: Impact of sugar concentration *in vitro* on photosynthesis and carbon metabolism during *ex vitro* acclimatization of *Spathiphyllum* plantlets. - Physiol. Plant. **96**: 298-304, 1996.