ACTA BIOLOGICA CRACOVIENSIA Series Botanica 42/1: 79–86, 2000

EFFECT OF CARBOHYDRATES ON CALLUS INDUCTION AND REGENERATION ABILITY IN *BRASSICA NAPUS* L.

HALINA BOGUNIA AND LESŁAW PRZYWARA

Department of Plant Cytology and Embryology, Jagiellonian University, ul. Grodzka 52, 31–044 Cracow, Poland

Received October 25, 1999; revision accepted March 28, 2000

The effect of 1%, 3% and 10% fructose, glucose, sucrose and ribose on callus induction and organogenesis was studied in *Brassica napus* L. cv. Evita. Hypocotyls and cotyledons of 7-day-old seedlings were used as explants. MS (Murashige and Skoog, 1962) was the basal medium. Calluses were produced from both types of explants in the presence of 2,4-D. There were significant differences in the frequency of callus induction between ribose and other sugars tested, as well as between low (1% and 3%) and high (10%) sugar concentrations. Irrespective of sugar type, callus induction was significantly lower on ribose- containing media and at high concentration. On hormone-free media, callus tissue formed very exceptionally and only from cotyledons. The amounts of callus tissue produced were highest on MS with glucose, followed by sucrose and fructose. In the regeneration experiments, explants were cultured on MS with 2,4-D as the sole growth regulator, and with NAA and kinetin. No regeneration occurred on medium with 2,4-D. In the presence of NAA and kinetin, organogenesis was observed only on media with 1% and 3% sugars, but on ribose the number of organs produced was very low. The highest regeneration ability occurred on sucrose-based medium.

Key words: *Brassica napus* L., carbohydrates, callus, regeneration.

INTRODUCTION

The importance of a carbon source in plant tissue culture has long been recognized. Sugars are necessary in living cells as a source of energy and carbon skeletons for biosynthetic processes. In cultured plant tissue a continuous supply of carbohydrates from the medium is necessary because the photosynthetic activity of in vitro tissues is reduced because of low light intensity, limited gas exchange and high relative humidity (Kozai, 1991). Carbohydrates are also necessary in tissue culture as osmotic agents. Recently it has become apparent that sugars are physiological signals repressing or activating plant genes involved in many essential processes, including photosynthesis, glycoxylate metabolism, respiration, starch and sucrose synthesis and degradation, nitrogen metabolism, pathogen defense, wounding response, cell cycle regulation, pigmentation and senescence (for details: Jang et al., 1997).

The role of sugar in organogenesis is important, but precise data are rather scarce. Millam et al. (1992) reported that the type of organogenesis in flax can be controlled by the type of carbohydrate used in the medium. In a comparison of maltose, sucrose and cellobiose, mean shoot regeneration was highest on medium containing 87 mM maltose, whereas root formation was most stimulated on medium with 87 mM sucrose.

Sugar concentration controls regeneration in sunflower. Bronner et al. (1994) found that, depending on the concentration of sucrose, either direct organogenesis or direct embryogenesis can be induced in the same hypocotyl zones of immature zygotic embryos. Shoots were induced at a concentration of 87 mM (3%) sucrose, whereas somatic embryos were formed at 350 mM (12%) sucrose. The experiments indicated that minimum threshold levels of both sugar supply and osmotic pressure were required for somatic embryogenesis, but not organogenesis, to occur. The nature of the sugar used

PL ISSN 0001-5296

was less important. Later, Jeannin et al. (1995) showed decreased frequency of organogenetic events parallel to increasing sugar concentration in sunflower. Experiments with media at constant sucrose concentration but increasing osmotic pressure confirmed that the osmotic pressure was responsible for the reduction in shoot number.

The capacity of plant tissue to utilize carbohydrates varies among species and explants, and depends on the capacity to absorb and metabolize the specific carbon source (Mezzetti et al., 1991). The most commonly used carbohydrate for plant tissue culture is sucrose. Sucrose is assumed to be the best carbohydrate because it is the main transport form of carbohydrate in most species. However, in some cases, sucrose can act as an inhibitor (Genga and Allavena, 1991). Despite the widespread use of sucrose, other sugars have also been reported as suitable carbon sources for tissue culture of different species and explants.

This paper reports a study of the effects of different sugars on callus production and regeneration in *B. napus* L. cv. Evita. The data are preliminary. Further experiments on other genotypes are necessary.

MATERIALS AND METHODS

Seeds of *B. napus* L. 'Evita' were placed in a tea infuser and surface-sterilized by soaking in 70% ethanol for 1 min and in Ace commercial bleach (diluted 1:1 with distilled water) for 12 min, followed by three rinses with sterile distilled water. The seeds were then placed in 10 cm petri dishes on moistened sterile filter paper and incubated at 25±3°C in the light. Two types of explants were obtained from 7-day-old seedlings: pieces of hypocotyl (~5 mm in length) and cotyledons. The explants were cultured in 8 cm or 10 cm petri dishes containing 20 ml medium, sealed with parafilm.

The treatments consisted of varying types and amounts of sugars. The medium was supplied with either 1%, 3% or 10% (w/v) of fructose, glucose, ribose or sucrose (Sigma). This corresponds to 56, 167, 556 mM for glucose and fructose; 67, 200, 667 mM for ribose; and 29, 88, 292 mM for sucrose. The dextro-rotatory form of carbohydrate was used. The sugars were added to the medium before autoclaving. MS (Murashige and Skoog, 1962) was the basal medium. In the callus induction experiment the medium was supplemented with 1 mg l^{-1} 2,4-D or was hormone-free. In the regeneration experiments, medium with 0.5 mg l^{-1} NAA + 1 mg l^{-1} kinetin was used. The concentration of NAA and kinetin was chosen after a series of preliminary experiments. The medium was solidified with 0.7% Difco Bacto-Agar and adjusted to pH 5.8 with 0.1 N HCl or NaOH after the agar was dissolved and prior to autoclaving at 121°C and 108 kPa for 20 min. All cultures were grown under a 16-hour photoperiod provided by cool-white fluorescent tubes (60–90 μm photons $m^{-2}s^{-1}$) at 25±3°C. The cultures were repeatedly subcultured at 3-week intervals. Each treatment had six to eight explants per petri dish, two petri dishes and three replicates. MS medium without a carbon source was the control. No callus growth or regeneration was observed on sugar-free medium.

Callus tissue production was estimated from the increase of fresh weight (FW) in hypocotyl culture recorded from every subculture. The petri dishes containing medium and explants were weighed before and after passage. The FW increase was calculated from the weight differences. Because of the very low callus production on ribose-containing media, this sugar was omitted in the experiment. The experiment was conducted for six months.

The data obtained were statistically analyzed using the *t*-test for dependent samples (STATISTICA for Windows, release 4.3).

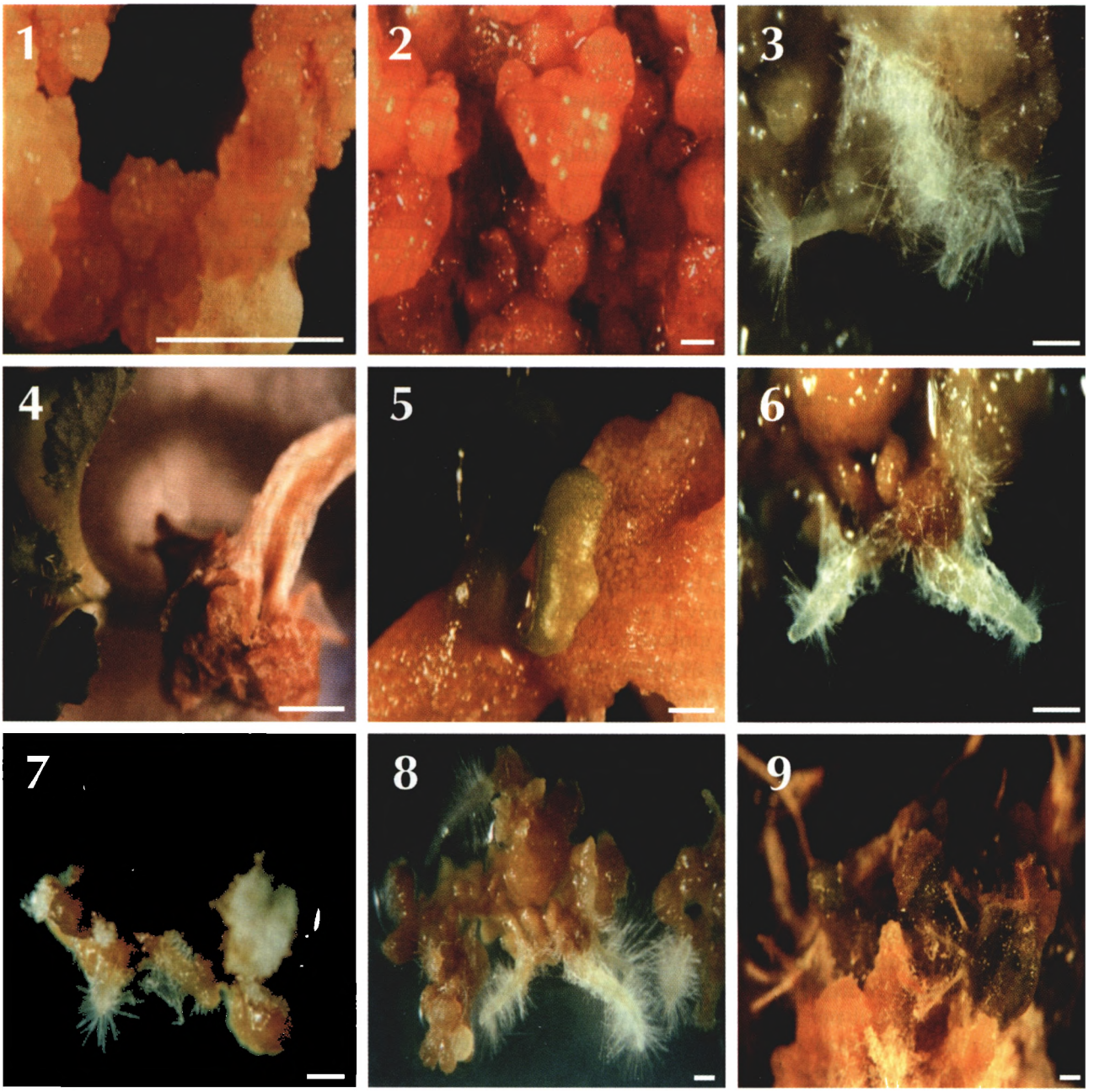
Microphotographs of in vitro cultures were taken under a Zeiss Stemi SV stereo microscope equipped with an MC 80 microphotographic attachment, on Kodak film.

RESULTS

EFFECT OF CARBOHYDRATES ON CALLUS INDUCTION

The frequency and time of callus induction, amounts of tissue produced (FW) and callus morphology were noted (Tabs. 1–3; Figs. 1–9). The frequency of callus induction was recorded after 3 weeks of culture. The effects of sugar type, sugar concentration, type of explant and auxin-sugar interaction on callus formation were analyzed.

Callus tissue production depended mainly on the presence of 2,4-D in the medium. On hormone-free media, callus formed very sporadically (3.2%). When auxin was present, calluses were produced on both types of explants and on all media, but there were differences resulting from sugar type and concentration (Tabs. 1, 2). The differences between type of explant were not significant. On ribose-containing medium, calluses were observed



Figs. 1–9. *Brassica napus* L. cv. Evita. Callus formation and morphogenetic response of explants obtained from 7-day-old seedling. **Figs. 1, 2.** Callus formation on MS medium with 1 mg l^{-1} 2,4-D. **Fig. 1.** Cotyledon; glucose 3%. **Fig. 2.** Cotyledon ribose 3%. **Figs. 3–9.** Organ formation on MS medium supplemented with 0.5 mg l^{-1} NAA + 1 mg l^{-1} kinetin. **Fig. 3.** Root formation on hypocotyl; glucose 1%. **Fig. 4.** Shoot formation on cotyledon; glucose 3%. **Fig. 5.** Shoot formation on hypocotyl glucose 3%. **Fig. 6.** Root formation on cotyledon; ribose 1%. **Fig. 7.** Root formation on hypocotyl; ribose 1%. **Fig. 8.** Root formation on cotyledon; ribose 1%. **Fig. 9.** Shoot and root formation on cotyledon; sucrose 1%. Bars on all figures = 1 mm.

on only 30% of the explants cultured, whereas callus tissue was produced on ~ 80–90% of those cultured on fructose-, glucose- and sucrose-enriched media. The differences between ribose and other sugars were statistically significant ($p<0.05$), and not significant ($p>0.05$) between glucose, fructose and sucrose.

Another very important factor influencing callus induction was sugar concentration. When carbohydrates were supplied at concentrations of 1% and

3%, calluses were produced on ~80–90% of the explants; at 10% concentration the number of explants with calluses was significantly lower (37.5%) (Tab. 2).

Irrespective of carbon source and concentration, calluses formed three weeks after the beginning of culture, except on medium with 10% fructose: in this case, callus appeared after 5–6 weeks.

Observations of callus morphology indicated that differences in callus features resulted rather from sugar concentration than from sugar type or explant type. When sugar was supplied at 1% concentration, light brown or yellow granular callus was produced. Media with 3% sugar stimulated a dark-brown granular or white nodular callus in most cases; at 10% concentration, dark-brown granular callus appeared, except on medium with fructose, where white granular callus was produced.

EFFECT OF CARBOHYDRATES ON HYPOCOTYL CALLUS PRODUCTION

The effect of carbohydrate source on the amount of callus tissue produced was studied only in hypocotyl culture. Media containing fructose, glucose or sucrose at 1% and 3% concentrations supplemented with 1 mg l^{-1} of 2,4-D were used. The experiment was carried out for about six months.

There were noticeable differences in the influence of carbon source on callus production. The highest FW increase occurred on media supplemented with 1% and 3% glucose, and the lowest on medium with 3% fructose. After six months of culture on 1% and 3% glucose, the FW increase was more than 8-fold, but on 3% fructose it was only doubled. On media with 1% and 3% sucrose, FW increased approximately 4-fold (Tab. 3). Statistical analysis showed significant differences between the three sugars used, but not between concentrations within particular sugars ($F = 24.688$; $df = 2.2$; $p < 0.05$). In most cases the highest FW increase was observed between the second and third months of culture; after 100 days there was a drastic decrease in callus production (Fig. 10).

EFFECT OF CARBOHYDRATE-GROWTH REGULATOR INTERACTION ON REGENERATION ABILITY

Cotyledons and pieces of hypocotyls were used in the regeneration experiments. For each treatment the number and type of organs formed were recorded (Tab. 4). Most frequently the organs were regenerated from callus. Generally, both types of organs were regenerated from the same explant.

TABLE 1. *Brassica napus* L. cv. Evita. Frequency (%) of callus induction on media containing different carbon sources

Carbon sources	MS	MS+2,4-D
Ribose	0.0	30.0a
Glucose	3.2a	90.0b
Sucrose	3.2a	83.3b
Fructose	3.2a	76.7b

Means in columns with the same letter are not significantly different at $p<0.05$. Each datum is the mean for the three concentrations tested, i.e., 1%, 3% and 10%.

TABLE 2. *Brassica napus* L. cv. Evita. Frequency (%) of callus induction on media versus sugar concentration

Sugar concentration	MS	MS+2,4-D
1%	0.0	82.5a
3%	2.5a	90.0a
10%	5.0a	37.5b

Means in columns with same letter are not significantly different at $p<0.05$. Each datum is the mean of the four sugars tested, i.e., ribose, glucose, fructose and sucrose.

TABLE 3. *Brassica napus* L. cv. Evita. Increase of fresh weight (FW) after 6 months of hypocotyl culture on MS medium with different sugars and 1 mg l^{-1} 2,4-D

Carbon source	%	g/day of culture
Fructose	121.0a	0.003a
Glucose	837.6b	0.020b
Sucrose	405.6c	0.009c

Means in columns with different letters are significantly different at $p<0.05$. Each datum is the mean for the two sugar concentrations tested, i.e., 1% and 3%.

Type of growth regulator and sugar concentration were found to be the main factors affecting regeneration ability. On the media with 2,4-D as the sole growth regulator, no regeneration occurred in any medium tested (data not shown). When the media contained 0.5 mg l^{-1} NAA and 1 mg l^{-1} kinetin, shoots and roots were produced in the presence of the four tested sugars (Figs. 3–9), but the number of organs produced greatly depended on carbohydrate type and concentration. On 1% and 3% fructose-, glucose- and sucrose-containing media the number of organs regenerated generally was markedly higher than on medium with 1% and 3% ribose, especially in the case of roots. Some differences in the influence of fructose, glucose and sucrose were statistically significant ($p<0.05$). Sucrose was most effective in inducing regeneration phenomena, as

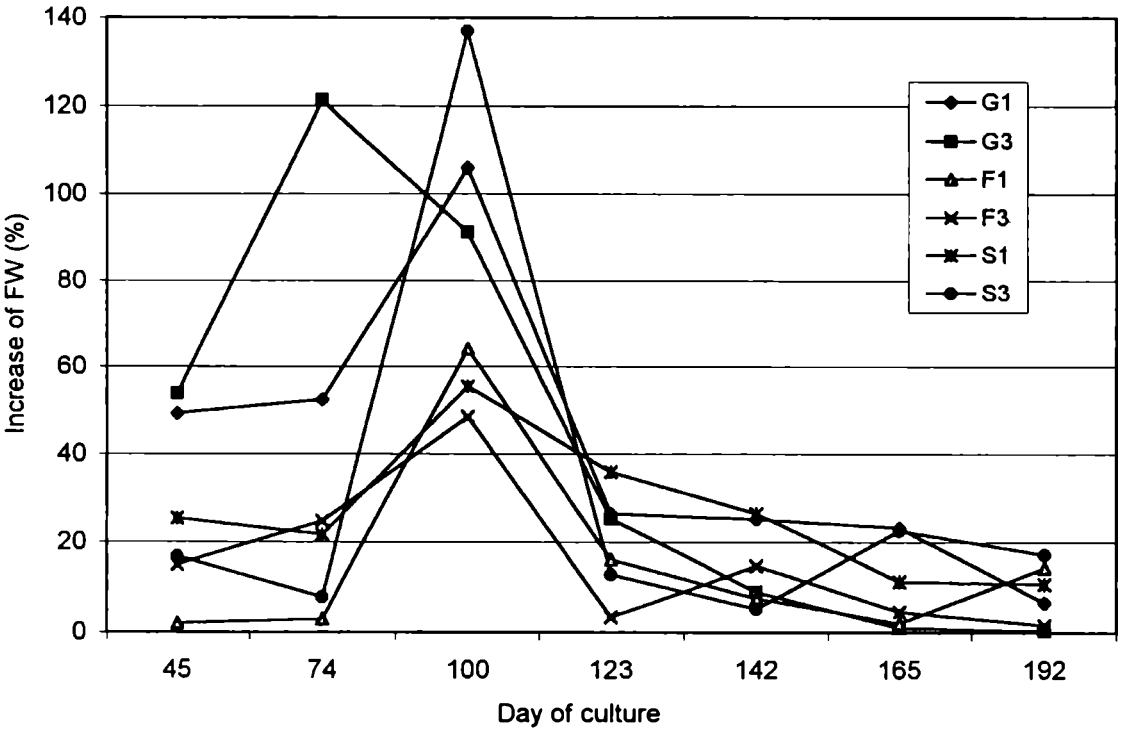


Fig. 10. *Brassica napus* L. cv. Evita. Increase of fresh weight (FW) during hypocotyl culture on MS medium with different sugars and 1 mg l^{-1} 2,4-D. G1, G3 – glucose 1% and 3%; F1, F3 – fructose 1% and 3%; S1, S3 – sucrose 1% and 3%.

was the 1% concentration; slightly higher numbers of organs were obtained from cotyledons. Regeneration did not occur at 10% concentration (data not shown).

DISCUSSION

Rogozińska and Drozdowska (1980) studied organ formation in rape tissue culture. They found that the presence of 2,4-D in the medium, though it promoted callus growth, inhibited root formation in culture of cotyledons. Regeneration of roots was achieved on media with NAA, IAA and IBA, and with combinations of these auxins with cytokinins. Shoot buds regenerated after about three weeks on media with NAA + BAP.

No systematic studies on the effect of carbohydrate source on tissue culture have been published for *Brassica napus*. This experiment addressed the influence of 1%, 3% and 10% concentrations of fructose, glucose, sucrose and ribose on callus induction and regeneration from hypocotyl and cotyledons of 7-day-old seedlings of *Brassica napus* L. cv. Evita. In the presence of 2,4-D, callus was induced from both types of explants cultured on MS medium; nevertheless, there were differences in the frequency of callus induction and the amount of callus tissue produced, resulting from sugar type and concentration. Glucose-, fructose- and sucrose-based media were significantly more effective in inducing callus than was ribose, as were lower concentrations (1% and 3%). These results agree with the experiments of Maataoni et al. (1998) on the influence of carbohydrates on callus culture in *Albizzia*. The sugars they tested were classified into four categories according to their capacity to induce cell proliferation: sugars without any callogenic effect which even proved toxic (arabinose and galactose), slightly callogenous sugars (xylose and lactose), moderately callogenous sugars (maltose) and highly callogenous sugars (sucrose, glucose or fructose). Histology confirmed that callogenous proliferation was organized in two different forms depending on the nature of the sugar. Explants cultured on fructose, glucose and sucrose produced very bulky calli which totally covered the explant after 20 days. Maltose and lactose induced callogenesis to a lesser extent. Callus proliferation under xylose was so weak that it could be observed only under a dissect-

TABLE 4. *Brassica napus* L. cv. Evita. Results of regeneration experiment on MS medium with different carbon sources and 0.5 mg l^{-1} NAA + 1 mg l^{-1} KIN

Carbon source*	Hypocotyl				Cotyledon			
	Shoots	Shoots/ explant	Roots	Roots/ explant	Shoots	Shoots/ explant	Roots	Roots/ explant
R1	1.3a	0.3a	3.3a	0.7a	2.7a	0.5ab	0.0	0.0
R3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G1	2.3ab	0.5ab	19.3c	3.9cd	2.3ab	0.5ab	24.3bcd	4.9bcd
G3	5.3abc	1.1ab	28.0cd	5.6cde	4.0ab	0.8ab	10.0a	2.0a
S1	5.7bc	1.1bc	33.7d	7.7e	8.7c	1.7c	55.3d	11.0de
S3	1.3a	0.3a	17.0c	3.4c	4.3bc	0.7ab	41.0c	8.2c
F1	7.7c	1.5c	34.3d	6.9de	4.7abc	0.9abc	32.0b	6.4b
F3	2.0ab	0.4ab	6.0b	1.2b	6.0bc	1.2bc	33.0bcd	6.6bcd

*R1, R3 – ribose 1% and 3%; G1, G3 – glucose 1% and 3%; S1, S3 – sucrose 1% and 3%; F1, F3 – fructose 1% and 3%. Means in columns with different letters are significantly different at $p<0.05$. Each datum is the mean of eight explants per petri dish, two petri dishes per medium, and three replicates.

ing microscope. Moreover, the response was delayed with the less callogenous sugars (xylose, maltose, lactose).

The similar results on the frequency of callus induction with sucrose, glucose and fructose might be connected with hydrolysis of the disaccharide sucrose to the monosaccharides glucose and fructose. Hisajima and Ito (1983) suggested that products of sucrose hydrolysis are used for energy production, formation of protein skeleton, and biosynthesis of new molecules of sucrose in cultured cells. According to Hagen et al. (1991), autoclaving can hydrolyse 5% of the sucrose in culture medium to glucose and fructose. Glucose is hydrolysed five times more quickly than sucrose (Hsiao and Bornman, 1991; c.f. Sawyer and Hsiao, 1992).

Mezzetti et al. (1991) reported an increase of sucrose level in explants of *Actinidia deliciosa* which was not correlated with the amount of sucrose in the medium. They suggested that sucrose synthetase present in kiwifruit cells was active in the synthesis of this carbohydrate. In our experiment the highest increase of fresh weight occurred on glucose-based medium. This suggests that glucose is more conducive to callus production than the other sugars tested.

The present data on the negative effect of high sugar concentration (10%) on callus production accord with other results. A number of authors have reported that a high level of sugar in the medium usually had an unfavorable influence on the culture. In wheat, optimal callus growth was observed at 2% sucrose (Galiba and Erdei, 1986); in soybean, callus induction occurred only at low concentration of sucrose (Komatsuda et al., 1991). Lemos and Baker (1998) found that callus appeared on *Annona muricata* L. explants cultured in media containing sucrose, glucose and galactose at 5 g l^{-1} to 30 g l^{-1}.

The role of sugar in morphogenesis is important but precise data are scarce. The different patterns of morphogenesis may be attributable to the type and concentration of carbohydrate. Several reports indicate that glucose is better for shoot proliferation, while sucrose is better for rooting. In *Solanum melongena*, when sucrose or glucose was supplied at 88 mM, glucose was more effective in inducing caulogenesis from leaf explants, but sucrose was better than glucose at 5.5 and 11 mM. At 88 mM sucrose (~3%) the number of shoots produced was about a third of what was observed at the optimal sucrose level. Moreover, 44 mM glucose yielded a significantly higher average number of shoots per explant than 22 mM sucrose. This indicates that the sugar in the medium has a limited osmotic role to play in caulogenesis, apart from being an energy source (Mukherjee et al., 1991). Similarly, in *Hyacinthus* the number of shoots was highest at low sucrose concentration (90 mM) and at moderate glucose level (180 mM) (Bach and Pawłowska, 1993). In cork oak, the highest number of shoots occurred on media with glucose and sucrose. Sorbitol and autoclaved fructose did not stimulate shoot proliferation. Glucose was also the most effective sugar for rooting promotion, followed by sucrose and filter-sterilized fructose. The best carbon sources during the proliferation and rooting phases of culture were 4% glucose and 3% sucrose, respectively. In cork oak, glucose combined with autoclaved fructose was less effective than glucose or sucrose, in terms of both rooting percentage and mean root number. The highest con-

centration of glucose and sucrose (6%) induced the highest fresh weight of plantlets. Shoot/root fresh weight ratio decreased with the increase in sugar concentration (Romano et al., 1995).

A continuous supply of sugar from the medium was necessary for root primordia formation and root development in apple (Pawlicki and Welander, 1995). Stem discs grown on medium without carbohydrates did not form any roots. Anatomical studies showed limited activity in the cambium, without formation of root primordia. When sucrose was used as the carbohydrate source, significant differences were obtained for the percentage of rooted discs and the number of roots per disc. Lower sucrose concentrations (29–59 mM) were more suitable for root formation than higher concentrations (88–76 mM). The use of glucose and fructose alone did not improve root formation, but at an equal molarity with sucrose (176 mM), combinations of glucose and sucrose increased the number of rooted discs and roots per disc, and decreased the percentage of discs with callus formation.

Auxin type, auxin concentration and carbohydrate are important factors affecting regeneration efficiency. In the present study, no regeneration was observed on media containing 2,4-D as the sole growth regulator. When NAA and kinetin were added to the medium, shoots and roots were produced, but there were differences in regeneration ability resulting from sugar type and concentration. On medium with 3% ribose (R3) no organs were formed, whereas on medium with 1% sucrose approximately ten organs were produced from each explant. Further studies to verify the influence of carbohydrates on different processes in vitro in *Brassica napus* are now in progress.

A significant interaction between sugar and auxin was investigated by Lazzeri et al. (1988) in soybean. The highest numbers of somatic embryos were produced on media containing combinations of low to intermediate levels of sucrose (1% or 2%) and NAA (6.25 or 12.5 mg l^{-1}). Loiseau et al. (1995) tested the effects of phytohormones, carbohydrates and amino acids on somatic embryogenesis in *Pisum sativum* and found that carbohydrate seemed to be a critical factor. Embryogenic efficiency and embryo development were promoted by high carbohydrate concentration. The best results were obtained with fructose (252–504 mM); the number of somatic embryos per cultured explant was 3- to 4-fold higher than the control (84 mM sucrose). Ashburner et al. (1993) studied the effect of NAA and sucrose levels on the development of cultured embryos of *Cocos nucifera*. Increasing sucrose concentration from 4% to 8% stimulated root elongation in the absence of NAA, and inhibited shoot growth whether NAA was present or absent. Application of NAA during the first four weeks of culture stimulated shoot growth, but it reduced shoot growth when the hormone was applied in the second and later months of culture.

REFERENCES

ASHBURNER GR, THOMPSON WK, and BURCH JM. 1993. Effect of α-naphthaleneacetic acid and sucrose levels on the development of cultured embryos of coconut. *Plant Cell, Tissue and Organ Culture* 35: 157–163.

BACH A, and PAWŁOWSKA B. 1993. Effect of carbohydrates in regeneration of *Hyacinthus orientalis* L. in long-term cultures. *Folia Horticulturae* 5: 3–11.

BRONNER R, JEANNIN G, and HAHNE G. 1994. Early cellular events during organogenesis and somatic embryogenesis induced on immature zygotic embryos of sunflower (*Helianthus annuus*). *Canadian Journal of Botany* 72: 239–248.

GALIBA G, and ERDEI LS. 1986. Dependence of wheat callus growth differentiation and mineral content on carbohydrate supply. *Plant Science* 45: 65–70.

GENGA A, and ALLAVENA A. 1991. Factors affecting morphogenesis from immature cotyledons of *Phaseolus coccineus* L. *Plant Cell, Tissue and Organ Culture* 27: 189–196.

HAGEN SR, MUNETA P, AUGUSTIN J, and LETOUMEAU D. 1991. Stability and utilization of picloram, vitamins and sucrose in tissue culture medium. *Plant Cell, Tissue and Organ Culture* 25: 45–48.

HISAJIMA S, and ITO T.1983. Activity and cellular distribution of disaccharides in cultured cells of Japanese Morning glossy. *Agr. Biol. Chem.* 47: 107–109.

JANG JC, LEON P, ZHOU L, and SHEEN J. 1997. Hexokinase as a sugar sensor in higher plants. *The Plant Cell* 9: 5–19.

JEANNIN G, BRONNER R, and HAHNE G. 1995. Somatic embryogenesis and organogenesis induced on the immature zygotic embryo of sunflower (*Helianthus annuus* L.) cultivated in vitro: role of the sugar. *Plant Cell Reports* 15: 200–204.

KOMATSUDA T, KANEKO K, and OKA S. 1991. Genotype X sucrose interactions for somatic embryogenesis in soybean. *Crop Science* 31: 333–337.

KOZAI T. 1991. Photoautotrophic micropropagation. *In Vitro Cellular and Developmental Biology Plant* 27: 47–51.

LAZZERI PA, HILDEBRAND DF, SUNEGA J, WILLIAMS EG, and COLLINS GB. 1988. Soybean somatic embryogenesis: interactions between sucrose and auxin. *Plant Cell Reports* 7: 517–520.

LEMOS EEP, and BAKER DA. 1998. Shoot regeneration in response to carbon source on internodal explants of *Annona muricata* L. *Plant Growth Regulators* 25: 105–112.

LOISEAU J, MARCHE C, and LE DEUNFF Y. 1995. Effects of auxins, cytokinins, carbohydrates and amino acids on somatic embryogenesis induction from shoot apices of pea. *Plant Cell, Tissue and Organ Culture* 41: 267–275.

MAATAONI ME, ESPAGNAC H, JABER B, and ALONSO-LOPEZ A. 1998. Regulation of in vitro callogenesis and organogenesis from *Albizzia* root explants by carbohydrate source modification. *Journal of Plant Physiology* 152: 494–501.

MEZZETTI B, CONTE LS, and ROSATI P. 1991. *Actinidia deliciosa* in vitro II. Growth and exogenous carbohydrates utilization by explants. *Plant Cell, Tissue and Organ Culture* 26: 153–160.

MILLAM S, DAVIDSON D, and POWELL W. 1992. The use of flax (*Linum usitatissimum*) as a model system for studies on organogenesis in vitro: the effect of different carbohydrates. *Plant Cell, Tissue and Organ Culture* 28: 163–166.

MUKHERJEE SK, RATHINASABAPATHI B, and GUPTA N. 1991. Low sugar and osmotic requirements for shoot regeneration from leaf pieces of *Solanum melongena* L. *Plant Cell, Tissue and Organ Culture* 25: 13–16.

MURASHIGE T, and SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497.

PAWLICKI N, and WELANDER M. 1995. Influence of carbohydrate source, auxin concentration and time of exposure on adventitious rooting of the apple rootstock Jork 9. *Plant Science* 106: 167–176.

ROGOZIŃSKA JH, and DROZDOWSKA L. 1980. Organogenesis and plant formation from cotyledon and callus culture of rape. *Acta Societatis Botanicorum Poloniae* 49: 5–20.

ROMANO A, NORONHA C, and MARTINS-LOUCAO. 1995. Role of carbohydrates in micropropagation of cork oak. *Plant Cell, Tissue and Organ Culture* 40: 159–167.

SAWYER H, and HSIAO KC. 1992. Effects of autoclave-induced carbohydrate hydrolysis on the growth of *Beta vulgaris* cells in suspension. *Plant Cell, Tissue and Organ Culture* 31: 81–86.