BIOLOGIA PLANTARUM (PRAHA)

22 (1) : 57–64, 1980

Utilization of Exogenous Sugars by Excised Maize Embryos in Culture

KORNÉLIA BURGHARDTOVÁ* and J. TUPÝ**

Institute of Botany, Slovak Academy of Sciences, Bratislava* and Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha**

Abstract. Sucrose was markedly superior to fructose and glucose in promoting growth of plantlets from immature maize embryos. The elongation of roots is shown to be more sucrose dependent than that of shoots. On the other hand, the exogenous sucrose was less effective than fructose as substrate for carbohydrate catabolism and for the synthesis of alcohol-insoluble compounds at the beginning of embryo cultivation. The absorbed fructose was found to be rapidly converted to sucrose and the level of endogenous sucrose derived from sugar supplied to the medium was higher in fructose- than in sucrose-fed embryos. The preferential utilization of fructose over sucrose, however, declined with the progress of germination which may be related to the decrease in proportion of scutellum in total mass and physiological activity of the embryo.

Isolated embryos of a number of plants grow better when supplied with sucrose than in the presence of glucose, fructose or of another sugar (for review see RAGHAVAN 1976). The superiority of sucrose in explant cultures is more general and it has been studied in detail especially in roots and in pollen. Some indications were obtained that the hight effectiveness of sucrose in promoting root growth may be related to a greater entry of this sugar into the conducting tissue and to a more rapid transport to the meristem (see STREET 1969). The respiration studies, however, failed to demonstrate a more rapid oxygen uptake by sucrose-fed roots (THOMAS *et al.* 1963). In pollen tubes studies on sugar uptake and metabolism indicated that sucrose is hydrolysed in the medium to fructose and glucose before it is utilized (TUPÝ 1962, NYGAARD 1977).

In the present paper we demonstrate that the utilization of externally applied sucrose by immature embryos at the beginning of their germination is much lower as compared with fructose, which is further shown to be rapidly converted to sucrose upon uptake. This finding is discussed in relation to the observed superiority of sucrose to hexose for growth of plantlets from the embryos.

Received August 9, 1979; accepted September 7, 1979

* *Present Address:* Research Institute of Petroleum and Hydrocarbon Gases, 823 11 Bratislava-Vlčie hrdlo, Czechoslovakia.

** *Address:* Flemingovo nám. 2, 160 00 Praha 6, Czechoslovakia.

MATERIAL AND METHODS

Plant Material

Embryos of *Zea mays* L. (line VIR-17) were excised from field grown plants 20 days after pollination. At this stage they were 3.5—4.0 mm long and had a diameter of 2.5—3.0 mm and their mean fresh and dry weight was 4.3 and 0.7 mg, respectively. For the experiments on sugar utilization the embryos were used either immediately after excision or following 3 days of cultivation when they had germinated. During this time the length of embryo axis increased nearly twice and the cells of both radicle and plumule manifested mitotic activity. The cells of the scutellum were enlarged and the greater part of their starch as well as that of the embryo axis disappeared.

Culture Conditions

The embryos were cultivated aseptically at 25 °C in the dark in White's liquid medium with sucrose or other tested sugar at 0.14 M concentration. The liquid medium was used instead of a solid agar medium to facilitate the uptake of labeled sugars. In preliminary experiments the absence of agar did not affect embryo growth. In testing the growth effect of various sugars the embryos were placed on filter paper discs fixed on the surface of 10 ml of cultivation solution in test tubes 14 mm in diameter. In metabolic studies 6 embryos were incubated with 1 ml of medium in 25-ml Erlenmayer flasks with exchangeable side arm and a disc of Whatman No 1 filter paper at the bottom. During the cultivation the flasks were gently shaken on a water bath.

Analysis of Sugar Utilization

The embryos were incubated in the medium containing 1 μCi ml^{-1} of sucrose-U-^{14}C (320 mCi mmol^{-1}) or fructose-U-^{14}C (180 mCi mmol^{-1}) and unlabeled sucrose or fructose at 0.14 M concentration. At the end of incubation the embryos were washed with distilled water and ground in a mortar with cold 75% alcohol. The sediment obtained after centrifugation was reextracted twice with alcohol. The combined extracts were concentrated by evaporation under reduced pressure and their radioactivity determined by scintillation counting. The residue after alcohol extraction was incubated for 24 h with 0.5 ml of 0.1 M solution of hyamine hydroxide in methanol. Then the samples were diluted with 2.5 ml of methanol and 0.5 ml of 0.7% PPO in toluen was added for scintillation counting.

To estimate the utilization of exogenous sugars in carbohydrate breakdown, $^{14}CO_2$ liberated by embryos was trapped by 0.3 ml of 2 N NaOH in exchangeable side arm of cultivation flasks. At certain time intervals the side arm was replaced by another one and the radioactivity of the trapping solution was determined by scintillation counting (MURRAY 1971).

At the end of incubation the alcohol soluble compounds were extracted from the embryos as described above and fractionated by paper chromatography using Whatman No 1 paper and the method of VÍTEK (1964). The chromatogram was cut into 2-cm sections and their radioactivity determined in the presence of 0.7% PPO in toluen.

All radioactivity measurements were carried out in a Packard Tricarb liquid scintillation spectrometer.

RESULTS

Growth Effect of Various Sugars

On media containing sucrose, invert sugar, fructose or glucose the isolated immature embryos germinated on the third day and produced healthy seedlings. The growth activity of the plantlets was, however, greatly de-

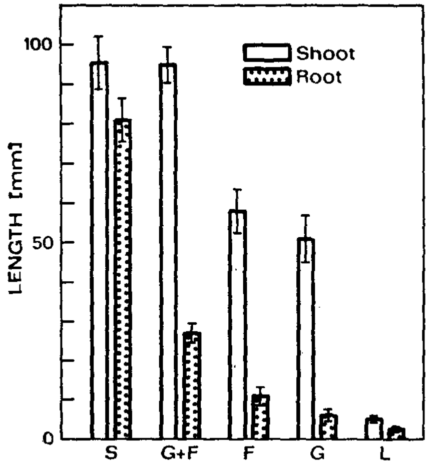

Fig. 1. Effect of carbohydrate nutrition of immature excised embryos on shoot and root growth. The embryos were cultivated for 24 days in White's liquid medium with sucrose (S), invert sugar (G + F), fructose (F), glucose (G) or lactose (L) at 0.14 M concentration. Means ± s.e. mean from ten seedlings.

pendent on the carbohydrate supplied. Best embryo growth was obtained in the medium supplemented with sucrose, following in order of decreasing effectiveness with glucose-fructose mixture, fructose and glucose (Fig. 1). The superiority of sucrose was much more marked in primary root elongation than in shoot growth. The shoot: root ratio increased with decreasing growth

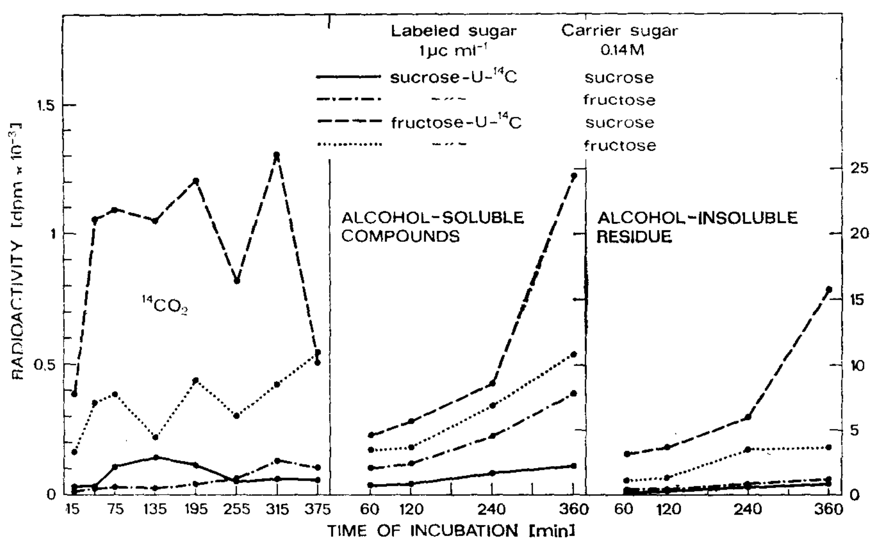

Fig. 2. Time course of utilization of exogenous sugars by excised embryos *in vitro*. The data on alcohol-soluble and -insoluble compounds indicate the radioactivity accumulated from the beginning of incubation, that on $^{14}CO_2$ correspond to the respective intervals. The $^{14}CO_2$ radioactivity of each time interval is expressed per 100 min of incubation.

TABLE 1

Comparison of exogenous sucrose and fructose utilization in freshly excised (A) and in 3 days-precultivated (B) embryos. (Radioactivity after 6 h of incubation)

Radioactivity [dpm mg^{-1} dry weight]		Radioactive and carrier sugar supplied			
		^{14}C-sucrose + sucrose	^{14}C-fructose + fructose	^{14}C-sucrose + fructose	^{14}C-fructose + sucrose
$^{14}CO_2$	A	288	1 386	232	3 560
	B	163	390	292	1 878
	B/A	0.6	0.3	1.3	0.5
Alcohol-soluble compounds	A	2 150	10 700	7 760	24 140
	B	4 170	11 470	14 840	20 770
	B/A	1.9	1.1	1.9	0.9
Alcohol-insoluble compounds	A	900	3 680	1 180	15 670
	B	890	2 590	2 270	5 860
	B/A	1.0	0.7	1.9	0.4
Total	A	3 338	15 766	9 172	43 370
	B	5 223	14 450	17 402	28 508
	B/A	1.6	0.9	1.9	0.7

activity of embryos from 1.2 on sucrose to 3.3, 5.2 and 8.4 on the three hexose containing media (means from two different experiments).

When lactose was used as carbohydrate source the germination of embryos was 1 day delayed, their further growth was very slow and on the eighth day they turned brown.

Comparison of Sucrose and Fructose Utilization

The utilization of externally applied sugar by immature embryos was followed in the course of 6-hour incubation. In order to eliminate the possible secondary effect of the physiological state of embryos in dependence on carbohydrate nutrition, the utilization of ^{14}C-labeled sugars was followed in the presence of both carrier sucrose and fructose.

In embryos taken immediately upon excision, the rate of $^{14}CO_2$ liberation from the exogenous sugars reached the highest level in 15 to 30 min and did not increase significantly during the further incubation (Fig. 2). It is shown to be greatly dependent on the nature of the carbohydrate supplied, being much higher on media with ^{14}C-fructose than in the presence of ^{14}C-sucrose. ^{14}C-fructose conversion to $^{14}CO_2$ further appeared to increase about 3 times when carrier fructose was replaced by sucrose.

The amount of alcohol-soluble labeled compounds grew continuously during the whole 6-hour incubation with ^{14}C-sugars (Fig. 2). At all time intervals their total radioactivity was the highest in the presence of ^{14}C-fructose-sucrose medium followed in decreasing order by ^{14}C-fructose-fructose, ^{14}C-sucrose-fructose and ^{14}C-sucrose-sucrose. The same order of effectiveness of the media compared was observed in the incorporation of radioactivity into the alcohol-insoluble compounds. Essentially same results as in Fig. 2

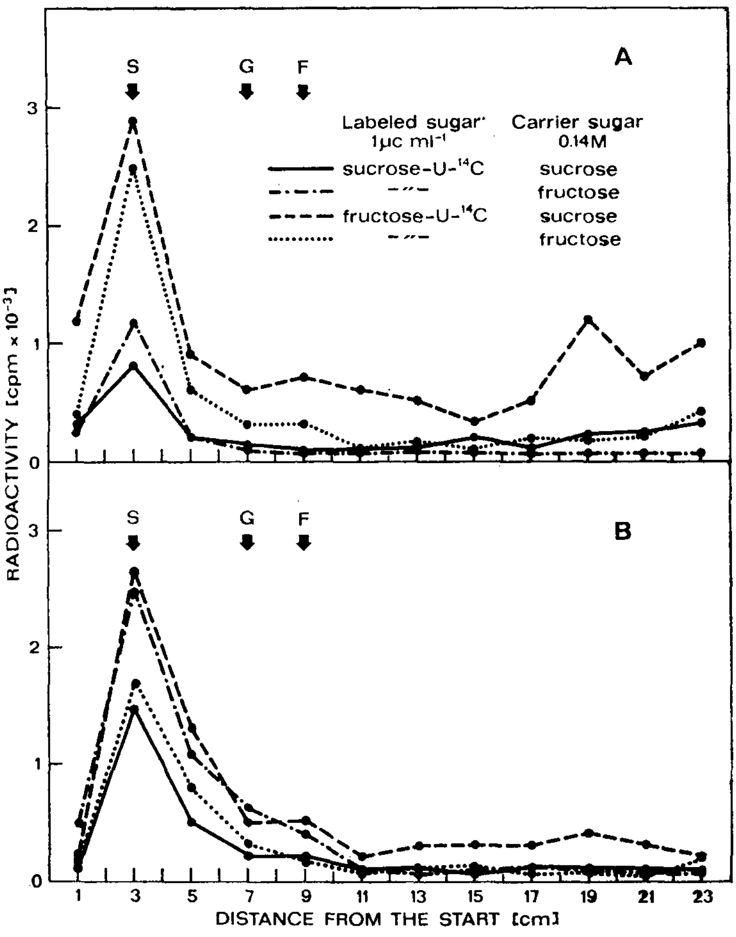

Fig. 3. Chromatographic analysis of alcohol-soluble fraction isolated from the embryos following 6 h of incubation with labeled sugars. The embryos were used for the experiment either immediately upon excision (A) or after 3 days of culture on the basal sucrose medium (B). The arrows indicate the position of sucrose, glucose and fructose.

were obtained with embryos taken following 3 days of cultivation in the basal sucrose medium. The total amount of sucrose absorbed was, however, markedly increased while that of fructose slightly diminished (Table 1). The higher label derived from ^{14}C-sucrose supplied with unlabeled sucrose was not observed in alcohol-insoluble fraction and in CO_2, which is most probably the result of important dilution with endogenous sucrose accumulated during the precultivation in sucrose medium. However, the ratio of ^{14}C-fructose : ^{14}C-sucrose derived radioactivity in the presence of homologous carrier sugars fell in the mean from 4.7 to 2.8 in all three groups of compounds analysed. An even higher decrease was observed when the carrier sugars were interchanged.

Analysis of Alcohol Extracts

The higher radioactivity of alcohol-soluble compounds from embryos incubated with ^{14}C-fructose suggested that fructose is taken up from the medium more rapidly than sucrose. To test this and to see the nature of the free carbohydrates present, the alcohol extracts were analysed chromatographically. The greater part of the radioactivity was detected in sucrose independently of whether the embryos were supplied with labeled sucrose or fructose (Fig. 3). In embryos incubated with labeled carbohydrates immediately upon excision (Fig. 3A), the order of sucrose radioactivity of the four samples analysed was the same as with the total radioactivity of alcohol extracts (see Fig. 2). In embryos that had germinated this order was changed in that the endogenous sucrose radioactivity in the ^{14}C-sucrose-fructose medium was higher than in ^{14}C-fructose with carrier fructose, being almost as high as in the presence of ^{14}C-fructose with unlabeled sucrose. Also the difference between ^{14}C-fructose and ^{14}C-sucrose supplied with homologous carrier sugars was much smaller than in freshly excised embryos (Fig. 3B), which confirms the results on alcohol-soluble fraction in Table 1.

DISCUSSION

The results on growth of the embryos on various sugar substrates confirm the often reported superiority of sucrose for explant cultures (*e.g.* STREET 1969, RAGHAVAN 1976). It is further shown that the immature maize embryos prefer invert sugar to glucose and fructose alone and fructose to glucose. There is, however, a marked difference in shoot and root response to carbohydrate feeding, the elongation of roots being much more depressed with decreasing effectiveness of sugar supplied for embryo growth. It suggests that the behaviour of embryos on the media tested may be primarily determined through the sugar effect on root development. On the other hand, no significant difference in growth depression of coleoptile and roots by hexose as against sucrose was observed in mature rye embryos (PURVIS 1944) and in submersion shaken cultures of wheat embryos fructose was the best carbohydrate for both shoot and root growth (BARCELLONA-VERO *et al.* 1968).

The greater part of the mass of embryo is formed by scutellum. It has been demonstrated that isolated maize scutellum readily absorbs glucose and fructose and converts them rapidly to sucrose (HUMPHREYS and GARRARD 1964, 1966). In our present experiments most ^{14}C of the alcohol-soluble fraction was found in sucrose, independently of whether the embryos were fed with labeled sucrose or fructose. However, in the presence of fructose the amount of externally derived sucrose was higher, which points out that fructose was taken from the medium more readily than sucrose. Correspondingly to this more ^{14}C from exogenous fructose appeared also in liberated carbon dioxide and in the alcohol-insoluble fraction. When ^{14}C-fructose was supplied with carrier sucrose and ^{14}C-sucrose with carrier fructose, the endogenous radioactivity increased in both cases but was again higher when fructose was the source of label. This indicated that the differences observed in the rate of sucrose and fructose uptake are not the result of some possible differences in the physiological state of the embryos in dependence on sugar nutrition. The positive effect of changing carrier sugar on labeled sugar utilization further points out differences in the mechanism of sucrose and fructose uptake and suggests that sucrose is taken up directly without inversion. This has been clearly demonstrated in mature scutellum slices in which evidence was obtained that sucrose is taken up actively and hexoses are taken by two processes, diffusion and active transport (WHITESELL and HUMPHREYS 1972). In the same work, higher gas exchange was observed in the presence of hexose than in the medium with sucrose which shows further similarity with the findings reported here on CO_2 evolution from exogenous sugars.

This similarity of our results with those obtained by others on scutellum slices suggests that especially in the freshly excised embryos the sugars were essentially taken up by the scutellum. This corresponds to the principal role of this organ in germinating seeds consisting in absorption of hexoses from the endosperm and in their transport in the form of sucrose to the embryo (EDELMAN *et al.* 1959). The rapid sucrose synthesis together with relatively low invertase activity in the scutellum further seems to be an important regulatory mechanism enabling the absorbed carbohydrates to be preferentially utilized by the embryo axis (BURGHARDTOVÁ and TUPÝ 1979). However, with the progress of germination the proportions in the mass and physiological activity change in favour of the developing seedling. In this relation, the present finding is of interest that in the embryo which had germinated, the utilization of externally supplied sucrose increased while that of fructose decreased. This could mean that the increasing participation of the radicle and later of roots on sugar uptake would progressively favour the utilization of exogenous sucrose over hexoses which could further be an explanation of the observed superiority of sucrose for growth of seedlings during the 24-days embryo cultivation. If this interpretation is correct and if there exists a positive relationship between carbohydrate utilization and embryo growth, it may be that initial feeding with fructose or invert sugar followed by cultivation on sucrose media could bring an improvement of the technique of embryo culture.

REFERENCE

BARCELLONA-VERO, L., MINO, L., ODDO, N., REALI-TEMPESTA, P., TONOLO, A.: Sterile submerged culture of some Graminaceous plantlets. — Experientia **24** : 1288—1289, 1968.

BURGHARDTOVÁ, K., TUPÝ, J.: Invertase of scutellum and embryo axis in maize embryos. — Biológia (Bratislava) **34** : 335—338, 1979.

EDELMAN, J., SHIBKO, S. I., KEYS, A. J.: The role of the scutellum of cereal seedlings in the synthesis and transport of sucrose. — J. exp. Bot. **10** : 178—189, 1959.

HUMPHREYS, T. E., GARDARD, L. A.: Glucose uptake by the corn scutellum. — Phytochemistry **3** : 647—656, 1964.

HUMPHREYS, T. E., GARRARD, L. A.: Leakage and storage of sucrose during sucrose synthesis in the corn scutellum. — Phytochemistry **5** : 653—663, 1966.

MURRAY, J.: Liquid scintillation counting of $^{14}CO_2$ in a toluene/triton X-100 system. — Int. J. appl. Rad. Isotop. **22** : 200—212, 1971.

NYGAARD, P.: Utilization of exogenous carbohydrates for tube growth and starch synthesis in pine pollen suspension cultures. — Physiol. Plant. **39** : 206—210, 1977.

PURVIS, O. N.: Studies in the vernalisation of cereals. VIII. The role of carbohydrate and nitrogen supply in the vernalisation of excised embryos of "Petkus" winter rye. — Ann. Bot. N.S. 8 : 285—314, 1944.

RAGHAVAN, V.: Experimental Embryogenesis in Vascular Plants. — Academic Press, London— —New York—San Francisco 1976.

STREET, H. E.: Growth in organized and unorganized system. — In: STEWARD, F. C. (ed.): Plant Physiology — A Treatise. Vol. 5B. Pp. 3—225. Academic Press, New York—London 1969.

THOMAS, D. R., CRAIGIE, J. S., STREET, H. E.: Carbohydrate nutrition of the excised tomato root. — Int. Soc. Plant Morphologists, Univ. of Delhi, Delhi 1963.

TUPÝ, J.: Radiorespirometric study of the utilization of exogenous sucrose, glucose and fructose by germinating apple pollen. — Biol. Plant. 4 : 69—84, 1962.

VÍTEK, V.: Sugar-like, diphenylamine-positive metabolites in pea seedlings. — Biochim. biophys. Acta 93 : 429—432, 1964.

WHITESELL, J. H., HUMPHREYS, T. E.: Sugar uptake in the maize scutellum. — Phytochemistry 11 : 2139—2147, 1972.

BOOK REVIEWS

WEBERLING, F., SCHWANTES, H. O.: PFLANZENSYSTEMATIK. Einführung in die Systematische Botanik. Grundzüge des Pflanzensystems. Dritte, neubearbeitete Auflage. Uni-Taschenbücher 62. — Verlag Eugen Ulmer Stuttgart 1979. 395 S. 116 Abb. DM 26,80.

Dieses Taschenbuch ist schon in Biologia Plantarum **15** (4) : 302, 1973 von mir besprochen worden. Die dritte Auflage ist neu bearbeitet worden: einige Passagen sind erweitert oder umgeändert worden, um der Weiterentwicklung der wissenschaftlichen Erkenntnisse seit der ersten Auflage (1972) gerecht zu werden. So z.B. die veränderte Darstellung des Entwicklungsganges verschiedener Algen- und Pilzgruppen, Herausstellung der Hornmoose als eigene Klasse der Bryophyten, erweiterte Ausführungen über Variabilität und Artbildung, geschlechtliche Fortpflanzung und vegetative Vermehrung der Pflanzen usw. Dieser Ausgabe wurden 12 neue Abbildungen hinzugefügt. Dementsprechend wurden Literaturverzeichnis und Namen- und Sachregister korrigiert und ergänzt. Und so wird diese Ausgabe des Kurzlehrbuches der Pflanzensystematik bestimmt vielen weiteren Studenten und Lesern aus botanischen und verwandten Disziplinen zum treuen Begleiter werden.

INGRID TICHÁ (*Praha*)

WALTER, H.: ALLGEMEINE GEOBOTANIK. Eine kurze Einführung. Zweite, verbesserte und ergänzte Auflage. Uni-Taschenbücher 284. — Verlag Eugen Ulmer Stuttgart 1979. 260 S. 135 Abb., 22 Tab. DM 19,80.

Die erste Auflage des vorliegenden Taschenbuches (1973) ist in Biologia Plantarum **16** (3) : 167, 1974 besprochen worden. Schon nach sechs Jahren erscheint die zweite Auflage, die bis auf einige Kleinigkeiten im Text und Abbildungen, Ergänzungen im Register der wissenschaftlichen Gattungsnamen und dem Verzeichnis der weiterführenden Literatur nicht wesentlich verändert worden ist. Der letzte Abschnitt des IV. Teiles „Die Eingriffe des Menschen in die Biogeozöne" wurde durch „Schlussbetrachtungen" ersetzt, wobei auf S. 237 der lebende Kolumnentitel wohl irrtümlicherweise noch stehen geblieben ist. Nichtsdestoweniger bezeugt die nach kurzer Zeit notwendig gewordene zweite Auflage am besten die hervorragenden Qualitäten dieser Einführung in die Geobotanik.

INGRID TICHÁ (*Praha*)