Phytochemistry, 1965, Vol. 4, pp. 919 to 924. Pergamon Press Ltd. Printed in England

DEVELOPMENT CHANGES IN THE DISTRIBUTION OF CARBOHYDRATES IN THE MAIZE ROOT APEX*

JANINA H. ROGOZINSKA,† PATRICIA A. BRYAN‡ and W. GORDON WHALEY

The Plant Research Institute, The University of Texas, Austin, Texas

(*Received* 14 *November* 1963)

Abstract—Qualitative and quantitative analyses of glucose, fructose, sucrose and starch were made on the first ten serial 1 mm segments of 3-, 4- and 5-day-old primary roots of *Zea mays*. On both a per segment basis and a per microgram dry weight basis glucose and fructose increase up to the fifth or sixth segment. Increased concentration of glucose and fructose are concomitant with cell elongation. They accompany not only the period of marked cell elongation, but also the cell enlargement that proceeds in that part of the root in which cell division is the dominant activity. In this region the increase of glucose and fructose is, however, at a much lower rate than in the so-called elongation region. In the second segment the concentration per dry weight of sucrose is at its peak and it exceeds that of glucose or fructose. Basipetal to the second segment, in the region of cell elongation, glucose and fructose are present in increasingly larger quantities than sucrose. On a quantity per segment basis starch is at its peak in the second segment, but the data expressed as concentration on a dry weight basis show, and electron micrographs confirm (unpublished data from this laboratory), that starch is more prevalent in the root cap and in certain cells basipetal to the apical region than in the cells of the division zone of the root.

THE root apex has now become a classic experimental material for analyses of development and growth and associated biochemical activities. These developmental phenomena have been related to changes in protein, DNA, RNA, the activity of several enzymes and some carbohydrates. Interest in the energetics of development and growth indicates, however, a need for more knowledge of substrate distribution and metabolism in the zone of the principal detectable developmental changes.

There have been repeated qualitative and quantitative determinations of simple sugars and starch in roots.[1–3] Ramshorn[4,5] studied the distribution of sugars along the developmental axis of *Vicia faba* roots, and the relative utilization of glucose, fructose and sucrose in aerobic respiration and the Embden-Meyerhof pathway in the root apex. Jensen[6] found that the total carbohydrate and the hexose content of the cells of the first 2 mm of *Allium cepa* roots were directly proportional to cell volume, but that this direct relationship was not retained during elongation. Hellebust and Forward[7] have followed changes in glucose, fructose and sucrose as well as sucrase (vertase) activity in the root apex of maize (*Zea mays*), and found notable increases of sucrose, glucose and fructose for 3 mm from the apex basipetally. The complex relationships of these sugars indicate involvement of consideration in addition to the simple translocation and inversion of sucrose.

* This investigation was supported by research grant RG-7289, from the National Institutes of Health and Institute of International Education No. 1204-111.12.

† Present address: Institute of Dendrology, Polish Academy of Sciences, Kórnik near Poznań, Poland.

‡ Present address: Department of Bacteriology and Botany, University of Syracuse, New York.

[1] G. L. RYGG, *Plant Physiol.* **20**, 47 (1945).

[2] A. G. GAWADI, *Plant Physiol.* **22**, 438 (1947).

[3] I. A. A. NADA and A. RAFAAT, *Indian J. Agr. Sci.* **25**, 271 (1955).

[4] K. RAMSHORN, *Naturwissenschaften.* **19**, 445 (1960).

[5] K. RAMSHORN, *Plant Physiol.* (Translated from Russian) **8**, 29 (1961).

[6] W. A. JENSEN, *Plant Physiol.* **33**, 64 (1958).

[7] J. A. HELLEBUST and D. F. FORWARD, *Can. J. Botany* **40**, 113 (1962).

Here consideration is given to the quantitative distribution of sucrose, glucose, fructose and starch in 1-mm segments of the first 10 mm of the maize (*Zea mays*) root apex in relation to developmental changes of growth, division and differentiation.

RESULTS

The procedure used revealed substantial amounts of sucrose, glucose, fructose and starch. Their distribution per segment for roots 3-, 4-, and 5-day-old maize is shown in Table 1. In the first segment, values for sucrose, glucose and fructose are all low, and the differences are probably not significant except perhaps for the higher sucrose value in 5-day-old roots. In the second segment the amount of sucrose is at its peak and it substantially exceeds that of glucose or fructose. Basipetal to the third segment, glucose and fructose are present in significantly larger quantities than sucrose.

Glucose per segment increases up to the sixth millimeter. In 3-day-old roots there perhaps is a slight decrease beyond the seventh millimeter and then a further increase. In 4- and 5-day-old roots there is a more distinct drop beyond the first peak and then an increase to the tenth millimeter segment. Fructose reaches high values in the fifth, sixth and seventh segments in 3-day-old roots and then decreases. In 4- and 5-day-old roots it reaches a somewhat higher value and remains high but perhaps not constant in more basipetal segments. After reaching a distinct peak in the second millimeter segment in 3-day-old roots sucrose decreases to a lower value. Sucrose distribution in 4- and 5-day-old roots is similar to that in 3-day-old roots.

Starch reaches a peak in the second millimeter and then falls off to variable values in the more basipetal segments. As seedling development proceeds there is a general decrease in the starch values.

In Fig. 1 the same data are presented on the basis of carbohydrates per unit of dry weight per segment. The carbohydrate distribution on this basis is similar to that shown in Table 1,

TABLE 1. CARBOHYDRATE DISTRIBUTION IN 3-, 4- AND 5-DAY-OLD-ROOTS OF *Zea mays*

Days	Carbohydrate† (μg/segment)	Root segment* 1	2	3	4	5	6	7	8	9	10
3	Glucose	0·4	1·5	5·3	10·4	12·2	14·0	14·2	13·8	13·5	14·4
	Fructose	0·3	0·7	1·0	2·4	4·3	4·5	4·4	3·0	2·1	1·8
	Sucrose	0·1	2·9	1·2	0·8	0·5	0·5	0·4	0·4	0·4	0·7
	Starch	4·4	9·2	6·7	4·4	3·5	2·9	3·1	2·9	3·1	3·5
4	Glucose	0·3	1·0	3·0	7·4	10·0	11·9	11·2	10·2	10·3	12·4
	Fructose	0·3	0·7	0·7	2·0	4·3	6·4	6·0	5·0	6·1	5·5
	Sucrose	0·2	2·7	0·8	0·5	0·4	0·6	0·5	0·5	0·3	0·5
	Starch	2·6	5·7	4·9	2·9	2·3	2·0	2·2	1·7	1·5	1·5
5	Glucose	0·2	1·2	3·9	8·2	10·3	11·7	8·7	8·9	8·2	11·9
	Fructose	0·3	1·1	2·3	4·4	7·3	6·6	6·9	7·1	6·8	7·8
	Sucrose	0·7	2·9	1·2	0·8	0·8	0·7	1·0	0·7	0·4	0·3
	Starch	2·4	5·2	4·3	2·1	1·7	1·5	1·1	1·1	1·1	1·0

* 1-mm segments numbered from the root apex.

† Glucose, fructose and sucrose present in 80% ethanolic extracts determined chromatographically; starch was extracted from the residue with perchloric acid,[8] and determined by the anthrone method (see Experimental).

[8] R. M. MCCREADY, J. GUGGOLZ, V. SILVIERA and H. S. OWENS, *Anal. Chem.* 22, 1156 (1950).

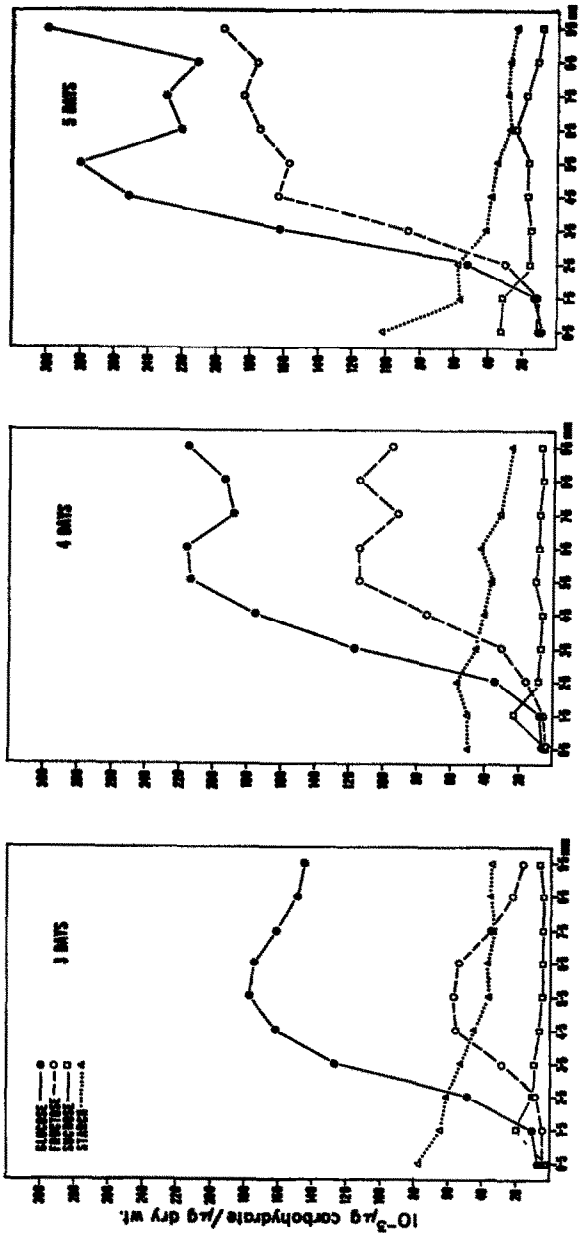
5 DAYS
4 DAYS
3 DAYS
SUCROSE
FRUCTOSE
DEXTROSE
STARCH
10⁻³µg carbohydrate /µg dry wt.

FIG. 1.

excepting that starch decreases from the first segment basipetally. The concentration values must be considered in relation to the changing weight per segment of the root apex that accompany progressive development of the seedling.

When these carbohydrates are considered on a per cell basis (for 5-day-old roots only, Fig. 2), glucose increases to the fifth millimeter, with an apparent drop in concentration in

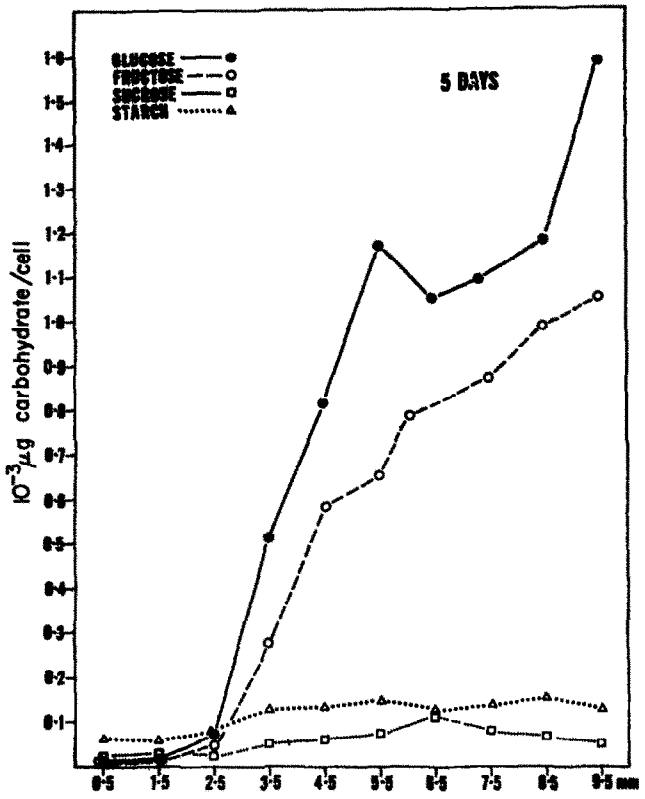

FIG. 2.

the sixth and seventh segments and a further increase to a higher peak. Fructose shows a similar increase but there is no drop. Sucrose is consistently low, with perhaps a slight increase in the sixth and seventh millimeters. Starch per cell appears constant from the root cap into the second millimeter segment after which a slight increase is indicated.

DISCUSSION

The experiments carried out by Edelman *et al.*,[9] with carbon-labelled sugars suggest that in germinating cereal grains glucose is absorbed from the endosperm by the scutellum where with fructose it is converted to sucrose and transported in this form. In extracts of barley scutella the authors demonstrated all the enzymes which can effect conversion of hexose to

[9] J. EDELMAN, S. J. SHIBKO and A. J. KEYS, *J. Exp. Botany.* **10**, 178 (1959).

sucrose via the pathway mediated by uridine-diphosphate glucose.[10,11] In maize, there is progressive decrease in the lipid content of the scutellum during germination[12] which could represent an additional source of sucrose.[13]

The high sucrose content in the second millimeter segment corresponds to the region of most intensive cell division[14] and the highest respiratory and fermentation activity.[4,15] Hellebust and Forward[7] indicated low sucrase (invertase) activity in this region, a fact that may account for the high concentration of sucrose here. The indicated peak of sucrose concentration does not appear when the data are presented on a per cell basis since there are approximately twice as many cells in the second segment than in the first.

Major increase of glucose and fructose coincide fairly closely with the extent of the region of major cell elongation. During this increase and subsequently throughout the first 10 mm, glucose predominates. This fact together with the low glucose and fructose concentrations of the apical portion of the root suggest that the high concentration of sucrose in the second segment is indeed an accumulation.

It is likely that the decrease in glucose in the basipetal region of the 3-day-old root apex, which becomes relatively more pronounced in the 4- and 5-day-old root apices and which also pertains on a per segment and per unit dry weight basis for fructose is associated with the development of secondary roots from the primary root. This dip in curve not only occurs in the region of secondary root origin but is moved acropetally in the aging root, commensurate with the acropetal progression of the origin of the secondary roots.[16]

The changing relations between the hexoses and sucrose and starch along the root axis suggest the possibility of uridine nucleotides participation in sugar conversion. Hydrolysis of sucrose and of starch surely accounts for some of the marked increase in glucose and fructose as cells are displayed basipetally with the starch hydrolysis making for some of the disparity between the hexoses. In the root apices of *Zea mays*,[7] sucrase activity exhibited one peak, coinciding with the region of maximum cell elongation, the region that is here reported as that of the greatest glucose and fructose increase.

In cross-section of root segments the amount of starch is very changeable. Thus, the average contents of starch per cell represented in Fig. 2 could be subject to error. The same may be true for other carbohydrates.

On a quantity per segment basis, the diminution of the difference is accounted for in part by reduction in the amount of glucose present, but more notably by an increase in the amount of fructose present. On a dry weight basis it is accounted for by an increase in the amount of fructose proportionately greater than the increase of glucose. Whether these results indicate differential increases or differential utilization is not known.

EXPERIMENTAL

Seeds of maize, a hybrid, University of Texas laboratory No. 854 × 857, 1960 stock, were grown in an incubator in red light at 25·0 ± 1·0° and ~80% relative humidity. The average root length of 3-, 4- and 5-day-old roots was 1·8, 7 and 10 cm respectively. Roots selected for analysis did not vary more than 10 per cent from these averages.

[10] L. F. Leloir and C. E. Cardini, *J. Biol. Chem.* **214**, 157 (1955).
[11] E. Cabib and L. F. Leloir, *J. Biol. Chem.* **231**, 259 (1958).
[12] L. D. Durr, III, *Dissertation*, University of Texas.
[13] H. Beevers, *Nature* **191**, 433 (1961).
[14] W. A. Stallard, *Dissertation*, University of Texas (1962).
[15] A. Rosenfield, *Dissertation*, University of Texas (1960).
[16] C. Heimsch, G. S. Rabideau and W. G. Whaley, *Am. J. Botany* **37**, 84 (1950).

At the end of the growth period, 10-mm apical segments were removed from the primary roots and cut with razor-blade cutters into 1-mm segments. The first segment included the root cap. Results are based on averages of three determinations each using 200–300 roots.

For the determination of cell number the root tips were incubated in 5% chromic acid for 24 hr then stirred vigorously with the "Vertex" mixer. Cell counts were made on the macerated material.

The root segments were killed in boiling 95% ethanol; sugars were then extracted by grinding with fine sand in 80% ethanol. The supernatant was evaporated in a vacuum oven under reduced pressure, at room temperature. The residue was dissolved in amounts of 80% ethanol corresponding to the fresh weight of the material. Quantities of extract corresponding to 5, 10 and 20 mg fresh weight were streaked on Whatman No. 1, chromatographic grade, paper. Chromatograms of standards and plant extracts were run four times with acetone:butanol:water (7:2:1) as the mobile phase.[17] The sugars were developed with a treatment involving anilinephthalic acid and differential heating.[18] The quantitative analyses were carried out with a Photovolt densitometer with filter No. 450, narrow band. Each spot was scanned and the minimum per cent transmission value recorded.[18] Quantities of sugars were determined by comparing these transmission values with curves from comparable densitometer readings of chromatograms prepared from known amounts of sucrose, glucose and fructose.

The residue remaining after the sugar extraction, was extracted twice with perchloric acid.[8] After filtering, residual glucose units were determined by means of the anthrone procedure,[19–21] using a Klett photoelectric colorimeter with No. 64 filter. The quantity of residual glucose units has been converted to starch equivalents according to the method of Pucher *et al.*[22]

[17] K. MACEK, *Handbuch der Papierchromatographie*, Vol. 1, p. 262. Fischer Verlag, Jena, (1958).
[18] R. J. BLOCK, E. L. DURRUM and G. ZWEIG, *A Manual of Paper Chromatography and Paper Electrophoresis*, (2nd ed.) p. 710. Academic Press, New York, (1958).
[19] F. J. VILES, Jr. and L. SILVERMAN, *Anal. Chem.* **21**, 950 (1949).
[20] F. A. LOEWUS, *Anal. Chem.* **24**, 219 (1952).
[21] L. H. KOEHLER, *Anal. Chem.* **24**, 1576 (1952).
[22] G. W. PUCHER, C. S. LEAVENWORTH and H. B. VICKERY, *Anal. Chem.* **20**, 850 (1948).