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The carbohydrate distribution in maize root apex in early growth stages*

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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. In some regions along the axis, both cell enlargement and cell division occur. In others, cell elongation and differentiation dominate. The most apical portion of the root proper contains a group of cells designated by Clowes (1958 a and b) as the "quiescent centre" and shown by him and by Jensen (1955 a and b) to have distinctly low rates of metabolism.

There have been repeated qualitative and quantitative determinations of simple sugars and starch in roots (Rygg 1945; Gawadi 1947; Nada and Rafaat 1955). Ramshorn (1960) studied the distribution of sugars along the developmental axis of *Vicia faba* roots. He found largely sucrose in the first three 1 mm segments of the root tip; glucose and fructose occurred only in trace amounts. Basipetal to 3 mm, sucrose concentration decreased somewhat and then increased to a second peak in the 6th mm segment, then decreased once more. From the 4th mm segment basipetally, glucose and fructose increased rapidly. Further work of Ramshorn (1961) has dealt with the relative utilization of glucose, fructose and sucrose in aerobic respiration and the Embden-Meyerhof pathway in the root apex. Ramshorn concluded that glucose is used largely in biosynthesis, fructose is largely subjected to oxidative breakdown and sucrose is used in both processes. Jensen (1958) 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 (1962) have followed changes in glucose, fructose and sucrose as well as sucrase (invertase) activity in the root apex of maize, and found notable increases of sucrose, glucose and fructose for 3 mm from the apex basipetally. The complex relationships of

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these sugars indicate involvement of considerations in addition to the simple translocation and inversion of sucrose.

Here, consideration is given to distribution of sucrose, glucose, fructose and starch in the first 10 mm of the maize root apex in relation to developmental changes of growth, division and differentiation.

EXPERIMENTAL

Seeds of maize, a hybrid, University of Texas laboratory number 854×857, 1960 stock, were planted between layers of filter paper lining the walls of 1 liter beakers which contained approximately 300 ml of distilled water. The seedlings were grown in an incubator in red light at 25.0±1.0°C and ca 80% relative humidity. The average root length of 3 day old roots was 1.8 cm, 4 day old roots 7 cm, and 5 day old roots 10 cm. Roots selected for analysis did not vary more than 10% from these averages.

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 rootcap. During the cutting period (approximately 2—3 hours) the sections were maintained in weighing bottles in an ice bath to reduce evaporation prior to fresh weight determination and to limit metabolic changes. Results are based on averages of 3 determinations each using 200—300 roots. The dry weights were determined separately on 100 root samples which were dried at 80°C for 24 hours.

For the determination of cell number the root tips were incubated in 5% chromic acid for 24 hours then stired vigorously with the "Vertex" mixer. Cell counts were made on the macerated material using an improved Neubauer hemacytometr.

After fresh weight determinations, segments were killed in 3 ml boiling 95% ethanol; sugars were then extracted by grinding with fine sand in 3 ml of 80% ethanol using a mortar and pestle. The residue was removed by centrifugation. Transfer of material was facilitated by use of an additional 3 ml of ethanol. Further extractions failed to remove any additional sugars. 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 and used for chromatographic analysis. Using micropipettes, quantities of extract corresponding to 5, 10, 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 (Macek 1958). The reducing and nonreducing sugars were developed with a treatment involving anilinephthalic acid and differential heating (Block et al. 1958). The quantitative analyses were carried out with a Photovolt densitometer with filter No. 450, narrow band. Each spot was scanned and the minimum percent transmission value recorded (Block et al. 1958). Quantities of sugars were determined by comparing these transmission values with curves from comparable densito-

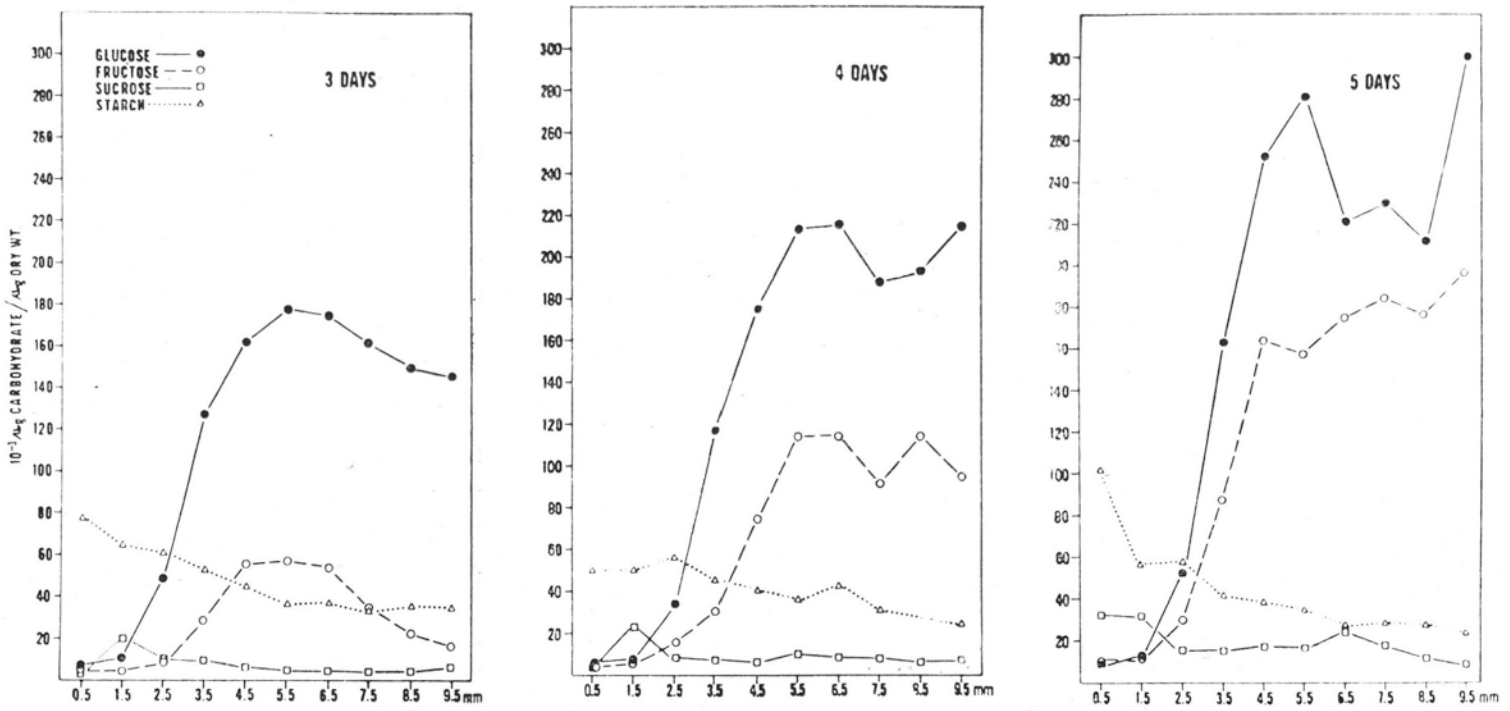

Fig. 1. Carbohydrate distribution on a dry weight basis per mm segment for 3, 4 and 5 day old roots

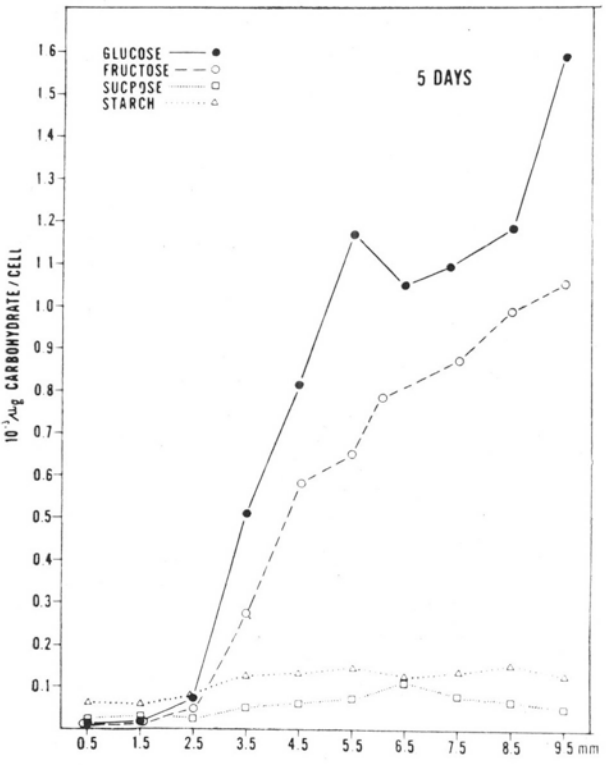

Fig. 2. Carbohydrate distribution on a per cell basis in successive 1 mm segments of 5 day old roots

meter readings of chromatograms prepared from known amounts of sucrose, glucose and fructose.

The standard error of the chromatographic estimations of sugars was 3,8%.

The residue remaining after the sugar extraction was extracted twice with perchloric acid (McCready et al. 1950). After filtering, residual glucose units were determined by means of the anthrone procedure (Viles and Silverman 1949; Loewus 1952; Koehler 1952), 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. (1948).

The standard error of the colorimetric estimations of starch was 3,1%.

RESULTS

The procedure used, revealed substantial amounts of sucrose, glucose, fructose and starch. Other sugars were absent, or present in non-detectable quantities.

The distribution of sucrose, glucose, fructose and starch per segment for roots 3-, 4-, and 5-days old is shown in Table 1. In the first mm 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 mm segment the amount of sucrose is at its peak and it substantially exceeds that of glucose or fructose. Basipetal to the 3rd mm, glucose and fructose are present in significantly larger quantities than sucrose.

Table 1
Carbohydrate distribution in 3, 4 and 5 day old roots

Days	µg/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

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 descends to 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 Figure 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, 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 (Table 2).

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 the 6th and 7th segments and a further increase to a higher peak. Fructose shows a similar increase but there is no drop, the increase continuing although at a lessened rate to the tenth millimeter. Sucrose is consistently low,

Table 2
Fresh and dry weights of successive 1 mm segments of 3, 4 and 5 day old roots

Days	mg fresh weight/segment									
	1	2	3	4	5	6	7	8	9	10
3	0.45	0.95	1.06	1.14	1.23	1.35	1.45	1.47	1.56	1.63
4	0.27	0.70	0.80	0.86	0.88	0.91	0.93	0.95	0.97	0.98
5	0.26	0.61	0.66	0.67	0.68	0.69	0.72	0.70	0.72	0.74

Days	µg dry weight/segment									
	1	2	3	4	5	6	7	8	9	10
3	57	141	110	83	76	79	82	86	90	99
4	50	113	86	63	57	56	52	54	53	58
5	23	92	73	50	45	42	40	39	39	40

with perhaps a slight increase in the 6th and 7th millimeters. Starch per cell appears constant from the root cap into the second millimeter segment after which a slight increase is indicated.

DISCUSSION

In seed germination and the initiation of seedling growth, reserve materials undergo enzymatic breakdown to simpler products. Some of these are transported as sugar to the growing regions of the developing seedling where they become available for use in biosynthetic processes and as energy sources.

The experiments carried out with carbon-labelled sugars by Edelman et al. (1959) on the role of the scutellum of cereal grains in the synthesis and transport of sucrose suggest that glucose is absorbed from the endosperm by the scutellum of the germinating grain, and, with fructose, converted to sucrose in the scutellum and transported in this form. In extracts of barley scutella the authors demonstrated all the enzymes which can effect conversion of hexose to sucrose via the pathway mediated by uridine-diphosphate glucose as described by Leloir and Cardini (1955) and Cabib and Leloir (1958). In maize, there is progressive decrease in the lipid content of the scutellum during germination (Dure 1960). This could represent an additional source of sucrose (Beevers 1961).

Sucrose is relatively constant in the more basipetal segments of the first 10 mm of the maize root. The region of high sucrose content in the 2nd mm segment corresponds to the region of most intensive cell division (Stallard 1962) and to the region of the highest respiratory activity (Rosenfield 1960; Ramshorn 1961) and fermentation (Ramshorn 1961). Hellebust and Forward (1962) 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 basic since there are approximately twice as many cells in the second mm segment than in the first.

Whereas glucose and fructose both increase somewhat in the first two segments (0 to 2 mm) major increases in these hexoses coincide fairly closely with the extent of the region of major cell elongation. Their predomination increase begin from the 3th segment. 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 2nd mm segment is indeed an accumulation.

It is likely that the decrease in glucose and fructose 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 is associated with the development of secondary roots from the primary root (Rogozińska et al. 1965).

The changing relations between the hexoses and sucrose and starch along the root axis confirm, that besides translocation and conversion the discussed forms of sugars are use in energetic and building processes. Sucrose is concentrated in the second mm and starch is built up in greater abundance in the cap then elsewhere. Hydrolysis of sucrose and of starch surely accounts for some of the marked increase in glucose and fructose as cells are displaced basipetally with the starch hydrolysis making for some of the disparity between the hexoses. Sucrase activity exhibited two peaks; at the beginning of the meristematic zone and maximum cell elongation of *Vicia faba* roots (Wanner and Leupold 1947). Hellebust and Forward (1962) have shown similar activity in the root apices of *Zea mays*, but they reported only 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. Hydrolysis of sucrose into glucose and fructose by sucrase has the equilibrium of the reaction so far to the side of hydrolysis that no detectable synthesis of sucrose can be brought about by this enzyme under physiological conditions (Hassid and Ballou 1957).

The differential increase that results in far more glucose than fructose accompanying cell elongation must relate, as Hellebust and Forward (1962) have noted, to metabolic reactions other than hydrolysis of sucrose. The role of uridine nucleotides in sugar conversion should also be considered.

In cross section of root segments the amount of starch is very changeable. Thus, such average per cell contents of starch as represented in Figure 2 have some error. The same can be thruth for other carbohydrates.

The decrease in the concentration of starch and sucrose from the second mm segment basipetally, can be partially the result of their conversion to glucose and fructose. Whether these results indicate differential increases of glucose and fructose due to starch and sucrose or differential utilization is not known.

SUMMARY

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 basic and a per microgram dry weight basic glucose and fructose increase up to the fifth

or sixth mm. The variation in glucose and fructose in basipetal portion of the curves probably indicate the initiation of secondary roots.

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 mm segment the amount and concentration per dry weight of sucrose is at its peak und it exceeds that of glucose or fructose. Basipetal to the second mm, 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 2nd mm segment, but the data expressed as concentration on a dry weight basis show, that starch is more prevalent in the first segment (including the root cap) and in certain cells basipetal to the apical region, than in the cells of the division zone of the root.

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Rozmieszczenie cukrowców w wierzchołku korzenia kukurydzy w początkowej fazie jego rozwoju

Streszczenie

Oznaczono jakościowo i ilościowo glukozę, fruktozę, sacharozę i skrobię w pierwszych dziesięciu, jednomilimetrowych segmentach korzeni kukurydzy. Analizy cukrów przeprowadzono na trzy, cztero i pięciodniowych pierwotnych korzeniach. Dane wyrażone zarówno w odniesieniu do segmentów, jak i w odniesieniu do suchej masy wykazują, że zawartość glukozy i fruktozy wzrasta aż do piątego lub szóstego segmentu, po czym opada. W cztero i pięciodniowych korzeniach zawartość glukozy i fruktozy wzrasta ponownie w segmentach końcowych.

Wzrost stężenia glukozy i fruktozy obejmuje strefy, w których zachodzi podział, powiększanie i wydłużanie komórek.

W segmencie drugim, stężenie sacharozy w odniesieniu do suchej masy jest największe i przewyższa ilościowo glukozę i fruktozę. W następnych segmentach, w strefie wydłużania komórek, glukoza i fruktoza obecne są w znacznie większych ilościach niż sacharoza. Maksymalna ilość skrobi (na segment) przypada w segmencie drugim, jednakże dane wyrażone w odniesieniu do suchej masy wykazują, że skrobia przeważa w pierwszym segmencie (obejmującym również czapeczkę korzeniową), a nie w komórkach strefy podziałowej korzenia.

Przeprowadzone badania wiążą metabolizm cukrowcowy z procesami wzrostu i rozwoju korzenia.