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Sugar Utilization by Developing Wild Type and *Shrunken-2* Maize Kernels[1]

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ABSTRACT

To characterize the movement of sugars during kernel development in maize, a newly devised *in vitro* kernel development scheme was utilized. Viable seeds of wild type maize (*Zea mays* L.) as well as the mutant *shrunken-2* (*sh2*) were found to mature when grown in culture with reducing sugars or sucrose as the carbon source. However, wild type and *sh2* kernels had greater germination, starch content, and seed weight when sucrose, rather than reducing sugars, was the carbon source. By the use of labeled sucrose it was shown that sucrose can move into endosperm tissue without intervening degradation and resynthesis. These results show that when grown *in vitro* the maize seed can utilize reducing sugars for development, but it prefers sucrose.

Kernel development of maize has received attention by workers in various areas. Starch synthesis has been of interest since there exist mutations affecting various steps along the biosynthetic pathway. Although it is quite clear that sucrose is a major sugar translocated to the developing ear (14) and that sucrose is found and even accumulates in the developing endosperm, studies have suggested that sucrose may not move intact into the endosperm but rather may be degraded before entry into the developing kernel and then be resynthesized in the endosperm tissues (12, 14–17). To delineate the biochemical lesion associated with various starch mutants and to more fully describe starch synthesis and metabolism we initiated studies in which we asked whether kernels could, in fact, develop normally when they were grown on reducing sugars rather than on sucrose. To do this, we utilized the *in vitro* kernel development scheme originally described by Gengenbach (5) and as modified by us (2).

This method was used previously to examine the effect of temperature on seed development (10) and also to follow the uptake of metabolites (18). In a previous report (2) we showed that seeds of wild type maize and the mutants *shrunken-1* and *shrunken-2* could grow to maturity in culture when sucrose was the carbon source. Starch synthesis and germination of the resultant seeds were similar to those of seeds of the same genotypes grown on the plant. This indicates that *in vitro* development is a useful tool for studies of starch synthesis.

In the studies reported here, the *in vitro* development technique was used to examine the growth of kernels on various sugars as well as to analyze the uptake of labeled sucrose into endosperm tissue. It was found that kernels will develop and are able to germinate when grown in culture with reducing sugars as the carbon source. The level of starch and germination percentages are altered, however, when compared to kernels grown on sucrose. Furthermore, there is an association between starch content and total germination in the kernels grown *in vitro*. Finally we show, by use of [^{14}C]sucrose, that sucrose can be transported into developing maize kernels grown *in vitro*.

MATERIALS AND METHODS

Plant Material. F_2 seed of W64A × 182A was the wild type maize (*Zea mays* L.) used in these experiments. The commercial cultivar 'Florida Stay Sweet' was the source for *sh2* maize.

All material was hand pollinated and harvested at 5 dpp[3]. Cob pieces with 12 kernels (6 kernels per row) were placed in culture as described previously (2). Sucrose, glucose, or fructose at 150 g/L was used as the carbon source. In addition, glucose plus fructose (75 g/L each) was used.

Germination and Starch Analysis. After 30 d in culture, the kernel blocks were removed, air dried, and the weights of individual kernels determined. Kernels were placed in moistened paper towels and incubated in darkness at 26°C for 1 week to determine total germination. Kernels were considered to have germinated if they showed root growth after 1 week. At least three replicates of 60 kernels or more were used for each determination.

To determine starch levels, kernels were ground in two volumes (w/v) of hot (65°C) 95% ethanol. The mixture was then filtered through Whatman No. 4 filter paper and washed with 95% ethanol. The residue was dried and ground to a powder. A 100 mg sample of the powder was solubilized in 10 ml of warm (about 45°C) 0.5 N NaOH while stirring followed by the addition of 70 ml of distilled H_2O. The pH of the solution was adjusted to 4.5 with acetic acid followed by addition of distilled H_2O to a final volume of 100 ml. Starch was hydrolyzed to glucose by the addition of 10 mg of amyloglucosidase to 1 ml of the above solution followed by incubation at 35°C for 1 h. Starch was then measured as free glucose using a Yellow Springs Instruments model 27 glucose oxidase analyzer.

Uptake of Radiolabeled Sucrose into Whole Kernels. Cob blocks with kernels of wild type and *sh2* were placed in culture on glucose as previously described (2). At 20 dpp, cob blocks were removed and divided to produce pairs of kernels with portions of the cob tissue (approximately 4 mm) still attached to the kernel base. Kernels were then transferred into fresh medium containing glucose with [U-^{14}C]sucrose (Amersham) (1 μCi/kernel, original specific activity = 253 mCi/mmol) which had been injected into slight depressions in the agar.

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[3] Abbreviation: dpp, days postpollination.

The kernels were placed into the depressions and, after a 1 h pulse in the [^{14}C]sucrose, transferred to fresh medium containing glucose as the carbon source. At hourly intervals kernels were harvested, the embryos and pericarps were removed, and the endosperms were homogenized in 1 volume (w/v) of 80% ethyl alcohol followed by centrifugation at 34,000*g* for 20 min. The supernatant was concentrated under a stream of air to remove the ethanol and then lyophilized. Four hundred μl of 80% (v/v) ethyl alcohol were added to each sample and 50 μl of the solution was spotted on Whatman No. 1 paper. Descending chromatography was carried out in a solvent system consisting of *n*-butanol:acetic acid:water (3:1:1, v/v/v) for 64 h. The chromatograms were cut into 1 cm squares, placed in scintillation fluid and monitored for radioactivity by scintillation spectrometry. The chromatography paper squares containing labeled sucrose were removed from the scintillation fluid, dipped twice in toluene and air dried. Sucrose was then eluted from the paper with water and lyophilized. One ml of a 0.05 M Na acetate buffer (pH 4.5) containing 200 units of invertase (Sigma) was added to the lyophilized sugar and the tube was incubated for 2 h at 35°C to hydrolyze the sucrose to reducing sugars. The mixture was then lyophilized, dissolved in 100 μl of 80% (v/v) ethyl alcohol and subjected to descending paper chromatography as described above. The areas corresponding to sucrose, glucose and fructose were removed and monitored for radioactivity.

MATERIALS AND METHODS

Growth of Kernels on Several Carbon Sources. Kernels of the two genotypes developed to maturity when placed in culture at 5 dpp on all carbon sources. Weights of the kernels are given in Table I. Wild type had greater weights than did *sh2*.

Kernels of wild type maize grown in the presence of the different sugars had the typical wild type phenotype at maturity. Possessing a dented crown which is characteristic of this wild type phenotype. Kernels grown on sucrose had a significantly greater weight than did kernels grown on the other carbon sources, indicating that sucrose was the superior carbon source at the concentration used (Table I).

Kernels of *sh2* grown in culture in the presence of the different carbon sources had their expected phenotype, which is characterized by a collapsed endosperm at maturity. Kernels produced on sucrose had a significantly greater weight in comparison to kernels grown on the other sugars indicating that, as in wild type, sucrose was the superior carbon source (Table I).

Germination and Starch Content of Kernels Grown in Culture. Wild type and *sh2* kernels grown on sucrose had greater germination than did kernels grown on glucose or fructose (Table I). The pattern seen for seed weight and germination was also observed for percent starch (Table I).

Table I. *Dry Weight, Starch Content and Percent Germination of Kernels Grown in Vitro on Different Sugars*

Genotype	Sugar Source	Dry Weight	Starch	Total Germination
		mg/kernel	*% dry wt*	
Wild type	Sucrose	195[a]	81[a]	96[a]
	Glucose	157[b]	68[b]	78[b]
	Fructose	126[b]	60[b]	75[b]
	Glu + Fru	139[b]	64[b]	77[b]
Shrunken-2	Sucrose	88[a]	32[a]	43[a]
	Glucose	68[b]	25[b]	36[b]
	Fructose	51[b]	22[b]	34[b]
	Glu + Fru	59[b]	28[b]	32[b]

[a,b] Difference at 5% probability level using Duncan's multiple range test.

Uptake of Labeled Sucrose. Analysis of the ethanol soluble extracts from developing endosperms by descending paper chromatography revealed the presence of labeled compounds that co-chromatographed with sucrose, glucose, and fructose (Figs. 1 and 2). Approximately 55% of the ^{14}C was found in the sucrose fraction at 0 and 1 h after the 1-h labeling in both genotypes (Figs. 1 and 2). Glucose and fructose together accounted for less than 40% of the labeled compounds during the same time

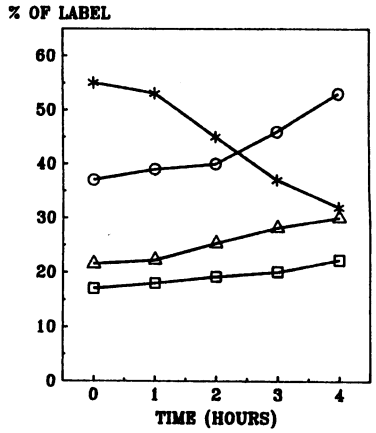

FIG. 1. Percentage of [^{14}C]sucrose (*), glucose (□), fructose (△), and total reducing sugars (○) extracted by ethanol from wild type endosperms over a 5 h period after exposure of kernels to [^{14}C]sucrose. Kernels were grown on sucrose and returned to sucrose after exposure to the label. Kernels were exposed to the labeled sugar for 1 h. Exposure to the label started 1 h before removal which is time 0.

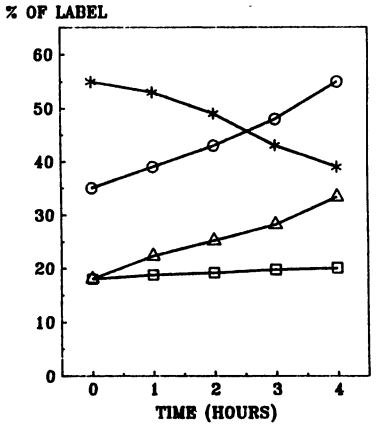

FIG. 2. Percentage of [^{14}C]sucrose (*), glucose (□), fructose (△) and total reducing sugars (○) extracted by ethanol from *shrunken-2* endosperms over a 5 h period after exposure of kernels to [^{14}C]sucrose. Kernels were grown on sucrose and returned to sucrose after exposure to the label. Kernels were exposed to the labeled sugar for 1 h. Exposure to the label started 1 h before removal which is time 0.

Table II. *Ratio of [^{14}C]Glucose to [^{14}C]Fructose of Sucrose Isolated from Endosperm Tissue*

Kernels were grown on glucose as described in text before transfer into [^{14}C]sucrose for the labeling period. Values are the averages of two experiments. The ratio of [^{14}C]glucose to [^{14}C]fructose of the supplied sucrose was 1.08.

Genotype	[^{14}C]Glucose/[^{14}C]Fructose
	ratio
Wild type	1.04
Shrunken-2	1.01

intervals (Figs. 1 and 2). From the 2nd to the 5th hour, the percent of label found in sucrose declined as the percent of label in reducing sugars increased (Figs. 1 and 2).

Previous experiments (1) showed that the ratio of glucose to fructose is approximately 1 when kernels are grown continuously on sucrose. This ratio is approximately 4 when kernels are grown on glucose. If this glucose and fructose serve as substrates for the eventual resynthesis of sucrose, then one would expect that the ratio of labeled glucose to fructose in the isolated sucrose would be altered by these pools and would reflect the dilution by the unlabeled glucose. As shown in Table II, the ratio of glucose to fructose in the isolated sucrose is the same. This suggests that sucrose was not hydrolyzed and resynthesized.

DISCUSSION

These experiments were done to ask whether kernel development of maize is dependent solely upon sucrose being the sugar source. While it is clear that sucrose can accumulate in the developing endosperm, especially in starch deficient mutants, elegant studies from Shannon and coworkers (4, 12, 14–17) have suggested that sucrose is hydrolyzed in the basal region of the kernel before transport into the endosperm tissue. After entry, sucrose may then be resynthesized. If reducing sugars can act as a source for the ultimate synthesis of sucrose in the maize endosperm, one might expect normal kernel development on reducing sugars. As shown here, the use of the *in vitro* kernel development technique allowed us to pose this question. It is clear from the data presented that although reducing sugars can serve as a carbon source, wild type and *sh2* kernels do prefer sucrose. This preference for sucrose was reflected in greater kernel dry weight, greater percentage of starch and greater total germination. It is known that in most tissue culture systems sucrose is the preferred carbon source (19) although in most cases the cultured tissue was callus. Here we are using a system where the tissue of interest, the kernel, is essentially intact with the cob tissues in contact with the media. Uptake of sugars must occur through the same tissues as in the intact plant.

One might hypothesize that the observed preference for sucrose, in comparison to reducing sugars, reflects the fact that sucrose is more efficiently taken up at the basal region of the kernel or the cob tissue. Such a scheme could then explain the data presented here and still include a sucrose→reducing sugar→ sucrose interconversion. That this is not the case is suggested by two different experiments. As shown here, when kernels are fed [^{14}C]sucrose, most label in the developing kernel is found in sucrose. With time, relatively more label is found in reducing sugars. Just the opposite would be expected if sucrose were being made at the expense of reducing sugars. Furthermore, the [^{14}C] glucose to [^{14}C]fructose ratio (Table II) in kernels grown on glucose is in keeping with the notion that the isolated sucrose had not been resynthesized from reducing sugars. If hydrolysis of sucrose occurred one would expect the ratio of the labeled glucose to fructose to be less than the supplied label since kernels grown on glucose contain approximately 4 times more glucose than fructose (1).

Results obtained by Jenner (8, 9) using techniques similar to those employed here suggested that sucrose is not hydrolyzed during uptake into developing wheat kernels. In an investigation of sucrose uptake into wheat kernels utilizing asymmetrically labeled sucrose in an *in vitro* system, Jenner determined that sucrose hydrolysis was not a prerequisite for transport into endosperm tissue (8, 9). The use of similar labeling techniques has shown that the transport of sucrose into potato slices (7), the phloem of beet (6), castor bean cotyledons (11), bean pod tissue (13), and carrot callus cultures (3) is accomplished without hydrolysis.

We conclude from all of these data that, while the developing corn seed has the ability to synthesize sucrose, complete degradation and resynthesis of sucrose is not requisite for normal kernel growth *in vitro*. Sucrose found in the endosperm can enter directly from the growth medium. It is possible that the observed sucrose had been degraded and resynthesized, but such reactions would have to occur in small, isolated pools of hexoses. Although this is conceivable, the data do not warrant such a complicated scheme and are compatible with the simpler conclusion that sucrose enters the endosperm without intervening reactions.

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