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# Transport and Metabolism of a Sucrose Analog (1′-Fluorosucrose) into *Zea mays* L. Endosperm without Invertase Hydrolysis[1]

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## ABSTRACT

**1′-Fluorosucrose (FS), a sucrose analog resistant to hydrolysis by invertase, was transported from husk leaves into maize (*Zea mays* L., Pioneer Hybrid 3320) kernels with the same magnitude and kinetics as sucrose. $^{14}$C-Label from [$^{14}$C]FS and [$^{14}$C]sucrose in separate experiments was distributed similarly between the pedicel, endosperm, and embryo with time. FS passed through maternal tissue and was absorbed intact into the endosperm where it was metabolized and used in synthesis of sucrose and methanol-chloroform-water insolubles. Accumulation of [$^{14}$C] sucrose from supplied [$^{14}$C]glucosyl-FS indicated that the glucose moiety from the breakdown of sucrose (here FS), which normally occurs in the process of starch synthesis in maize endosperm, was available to the pool of substrates for resynthesis of sucrose. Uptake of FS into maize endosperm without hydrolysis suggests that despite the presence of invertase in maternal tissues and the hydrolysis of a large percentage of sucrose unloaded from the phloem, hexoses are not specifically needed for uptake into maize endosperm.**

The lack of direct vascular or cellular connections between maternal and embryonic tissues in developing seeds and caryopses, necessitates passage of phloem-imported assimilates through the apoplast (for review see Thorne [14]). It has been suggested that in maize and sorghum the apoplast of the placenta and chalazal regions, which contain many dead cells, functions as a temporary storage site for sugars before absorption into the endosperm (2, 6). Evidence for this is as follows: (a) high hexose concentrations are found in the placental sac, for sorghum 300 to 400 mM (6), and for maize 470 to 800 mM of glucose equivalents (12); (b) there is a buildup of radioactivity in the pedicel of maize after a pulse of $^{14}CO_2$ to the leaf ear (2).

In maize (7, 10) and sorghum (6), incoming sucrose is inverted to hexoses in the placental-chalazal, pedicel, or basal endosperm regions during movement into endosperm tissues. In maize, the highest kernel activities of acid invertase are extracted from the placental-chalazal and pedicel tissue (11). Invertase hydrolysis may be needed to provide hexoses for carrier-mediated uptake into the endosperm, as is the case for uptake of hexoses into sugarcane stem cells (14).

Here we have used an analog of sucrose, FS,[3] which is a poor substrate for invertase (4, 8), to elucidate the role of invertase hydrolysis in uptake of phloem-supplied sucrose into maize endosperm. FS behaves similarly to sucrose in phloem-loading in leaf tissue (4) and in translocation in soybean and sugar beet plants (8). The $K_D$ for FS uptake by soybean cotyledon protoplasts is 0.9 mM compared to 2.0 mM for sucrose (4). FS is resistant to hydrolysis by invertase; the ratio of hydrolysis of sucrose to FS via yeast invertase is 4200, and hydrolysis of FS by crude extracts from developing soybean leaves or wheat germ is below measurement (8). In contrast, the ratio of breakdown of sucrose to FS by sucrose synthase from developing soybean leaves and wheat germ is 3.6 (8). Therefore, if invertase hydrolysis is necessary to provide hexoses for uptake into maize endosperm, FS should not enter the endosperm. If FS does enter the endosperm, the inversion to hexoses must not be a prerequisite for sugar entry, and once in the endosperm FS could be metabolized via sucrose synthase.

## MATERIALS AND METHODS

**Supply of $^{14}$C-Sugars.** *Zea mays* L. (Pioneer Hybrid 3320) was grown in a greenhouse with supplemental lighting. Cobs were removed from plants 20 to 22 d postpollination and the shank was recut and placed in water. The upper half of the cob was cut off and all but the two innermost husk leaves were removed. The cut cob and husk leaves were kept moist with wet cheesecloth covered by aluminum foil. Total sucrose and hexose levels in the endosperm remained at 15 to 30 μmol/g fresh weight and 9 to 20 μmol/g fresh weight, respectively, throughout the experiment, indicating that sugars were not depleted despite isolation of the cob from the plant. Transport studies were performed under a mixture of fluorescent and incandescent lighting at 380 μmol/$m^2 \cdot s$.

$^{14}$C-sugars were supplied to the phloem translocation path by entry into the transpiration stream via slits in the husk leaves, followed by loading into the phloem. [$^{14}$C]Sucrose or [$^{14}$C]glycosyl-FS was supplied in 20 mM cold sucrose with 2.1 GBq $^{14}$C-label/mmol sucrose. A total of 50 μl (2.1 MBq) of $^{14}$C-sugar was added in 5 μl droplets over a period of 1 h. The additions were made via three, 0.5 cm wide slits spaced 5 cm from each other and cut perpendicular to the vascular bundles. $^{14}$C-Sugars rapidly entered the transpiration stream such that 15 min after addition $^{14}$C-label was detected directly apical to the slits with Geiger-Mueller tube hand monitor. Sucrose without tracer was added to the slits for the duration of the experiment. Eight or four

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[3] Abbreviations: FS, 1′-deoxy-1′-fluorosucrose; MCW, methanol: chloroform:water.

kernels were harvested with time in descending rows taken directly underneath the husk leaf area supplied with $^{14}$C-sugar. The final Bq in one kernel was approximately 0.01% of the total Bq added.

Harvested kernels were frozen in dry ice and stored at −20°C. Kernels were dissected into endosperm, embryo, pericarp, and pedicel (basal maternal tissues), and the fresh weight of each measured. Embryos were digested overnight in NCS tissue solubilizer (Amersham) and analyzed by liquid scintillation counting.

Pedicel and endosperm tissues were ground with a Polytron homogenizer and extracted with MCW (13:4:3) following the procedure of Shannon (9). An aliquot of the dried MCW-insoluble pellet was digested as described above. Chloroform and methanol-water fractions were separated and the resulting methanol-water fraction was passed through 0.5 ml each of formate anion and $H^+$ cation resin (Bio-Rad). The columns were washed with water and the elutants were brought to dryness and redissolved in 90% methanol. An aliquot was acetylated and $^{14}$C-acetylated sugars were separated by TLC as described previously (8). Another sample was diluted, dried, and used for enzymic assay of sucrose and hexoses (8).

**Source of Chemicals.** [U-$^{14}$C]sucrose and [$^{14}$C]glucose were purchased from New England Nuclear. [$^{14}$C]Glycosyl-FS was synthesized as described previously (4, 8).

## RESULTS

[$^{14}$C]FS acted as a tracer of sucrose in translocation from the husk leaf to the kernels. Uptake of [$^{14}$C]FS and [$^{14}$C]sucrose into the endosperm and the pedicel followed the same kinetics (Fig. 1) and was of the same magnitude (Table I). The distribution of $^{14}$C-label between endosperm and pedicel tissue samples was also similar for both [$^{14}$C]FS and [$^{14}$C]sucrose additions (Fig. 2). The percentage of $^{14}$C-label in the embryos remained less than 5% and was similar for the two sugars.

FS was transported and arrived intact in the pedicel and passed into the endosperm as FS. At the end of an experiment with [$^{14}$C]FS, 89% of the total $^{14}$C-label in the husk leaf was in FS and 9% was in sucrose, indicating that there was only limited metabolism of FS before transport. In the pedicel, 1 h after the end of addition of [$^{14}$C]FS, 85% of the radiolabel in neutrals was in FS (compared to 26% in sucrose with [$^{14}$C]sucrose as tracer).

Though $^{14}$C-sugars were supplied over a period of 1 h, labeling patterns indicate that 3 to 4 h after addition entry of $^{14}$C-sugar reached steady state. After 3 to 4 h radiolabel in the pedicel stopped accumulating and total $^{14}$C remained relatively constant, indicating that entry of $^{14}$C-tracer equaled exit (Fig. 1A). In contrast, radiolabel continued to accumulate in the endosperm for 10 h after addition (Fig. 1B), as is expected for a terminal, storage pool. The existence of steady state labeling probably resulted from storage of radiolabel in the husk leaf followed by continued entry into the transport path. In the final sample, radiolabel in the pedicel was decreased and accumulation rate in the endosperm appeared to decline, indicating that arrival of radiolabel in the kernel was beginning to decline (Fig. 1).

The occurrence of steady state labeling was also seen in labeling patterns of sucrose, hexoses, and FS. When [$^{14}$C]sucrose was supplied, $^{14}$C in both hexoses and sucrose in the pedicel increased during the initial 3 h, then remained relatively stable with variation among sampling points (Fig. 3A). Radiolabel in sucrose in the endosperm increased throughout the 10 h experiment, with only low levels of $^{14}$C in hexoses (at most only 10–12% of total radiolabel) (Fig. 3B). The general pattern of labeling of sugars shown here is similar to that reported by Shannon (9, 10) in which kernels received $^{14}$C-assimilates from leaves fed $^{14}CO_2$, indicating that our method of adding sugars to the husk leaf resulted in metabolism of sucrose similar to supply from the leaf.

There was little metabolism of FS in the pedicel. [$^{14}$C]FS accumulated for 4 h in the pedicel and then remained at a steady level until the last sampling time (Fig. 4A). Labeled sucrose and hexoses (roughly 5 and 12% of total $^{14}$C in neutrals, respectively) probably resulted from synthesis of [$^{14}$C]sucrose from [$^{14}$C]FS in the husk leaf (9% of $^{14}$C was in [$^{14}$C]sucrose in the husk leaf at the end of the experiment). In the endosperm, FS continued to accumulate up to 6 h, and was readily metabolized and used in synthesis of sucrose (Fig. 4B).

Radiolabel from [$^{14}$C]sucrose accumulated in the MCW-insol-

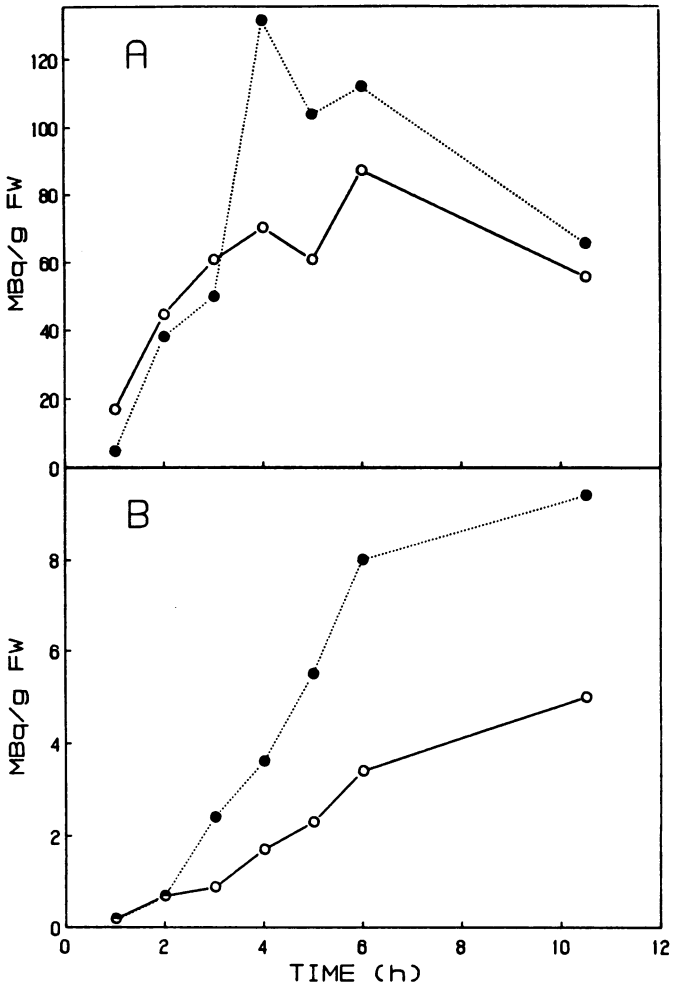


FIG. 1. Accumulation of $^{14}$C in maize pedicel (A) and endosperm (B) from [$^{14}$C]sucrose (○) or [$^{14}$C]FS (●) supplied to the husk leaf. Data for each sugar are from one of three experiments with similar results.

Table I. *Accumulation of $^{14}$C in Endosperm (MBq/g fresh weight) from [$^{14}$C]Sucrose and [$^{14}$C]FS Supplied to the Husk*

Accumulation of radiolabel from the two sugars is not significantly different for each time point at the 10% significance level using the Student's *t* test.

| Expt No. | Sugar | Time (h) | | | |
|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 10 |
| 1 | Sucrose | 0.18 | 0.88 | 3.46 | 5.04 |
| | FS | 0.06 | 2.54 | 8.56 | 10.16 |
| 2 | Sucrose | 0.32 | 1.07 | 3.26 | 4.00 |
| | FS | 0.09 | 1.59 | 3.86 | 2.75 |
| 3 | Sucrose | 0.08 | 2.39 | 2.84 | 6.12 |
| | FS | 0.02 | 0.34 | 2.36 | 7.04 |
| Mean | Sucrose | 0.19 | 1.45 | 3.19 | 5.05 |
| | FS | 0.06 | 1.49 | 4.93 | 6.65 |

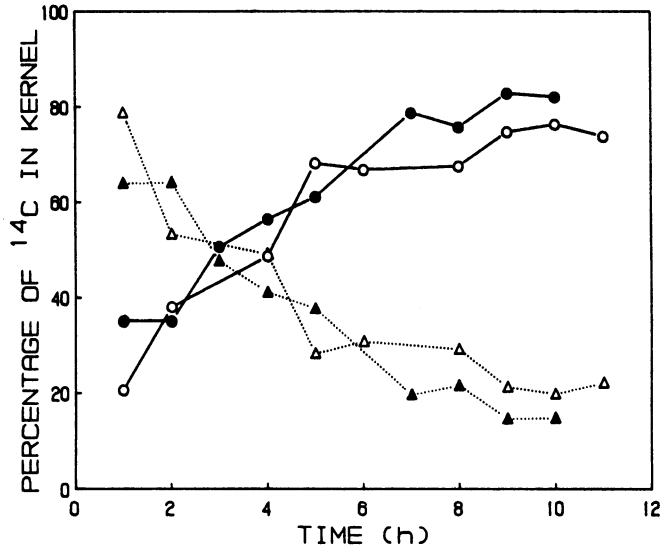


FIG. 2. Percent distribution of total $^{14}C$ among endosperm (○, ●), and pedicel (△, ▲) from [$^{14}C$]sucrose (open symbols) or [$^{14}C$]FS (closed symbols) supplied to the husk leaf. Data for each sugar are from one of three experiments with similar results.

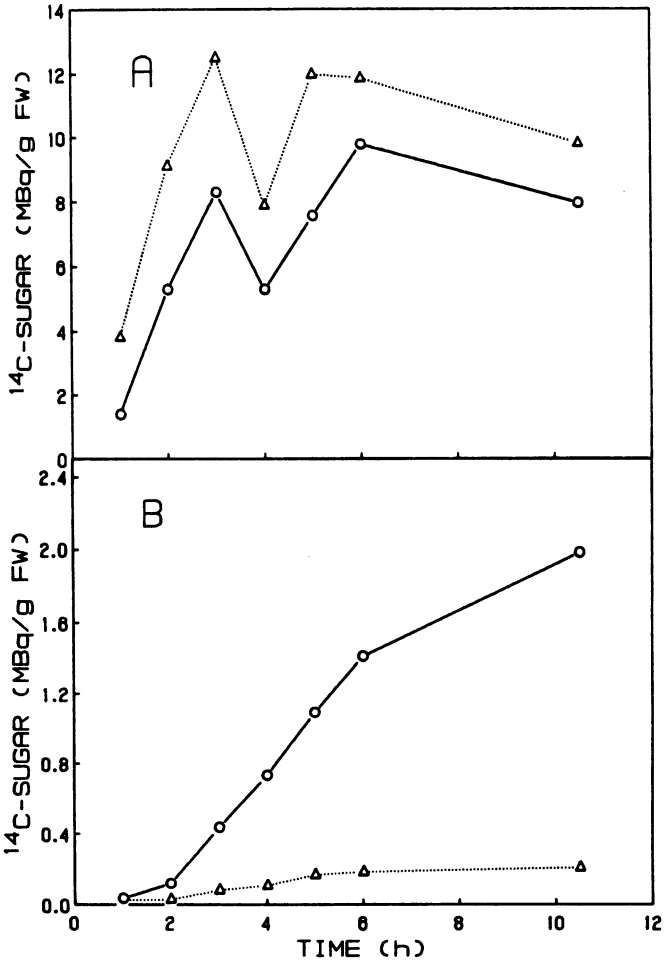


FIG. 3. Accumulation of $^{14}C$ in sucrose (○) and hexose (△) of neutral fraction of pedicel (A) and endosperm (B) from [$^{14}C$]sucrose supplied to the husk leaf. Data are from one of three experiments with similar results.

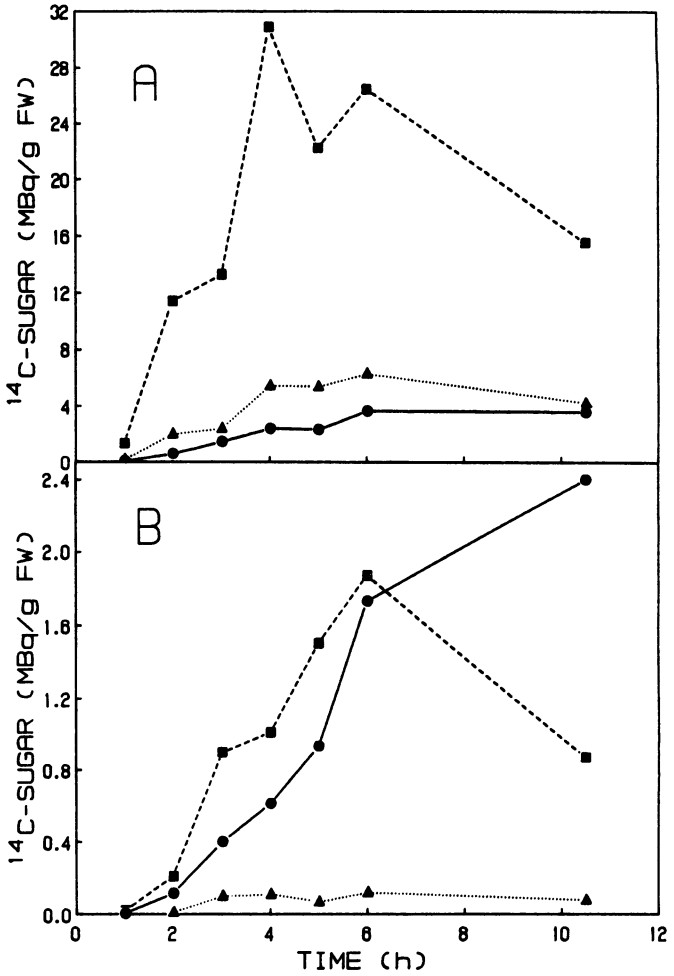


FIG. 4. Accumulation of $^{14}C$ in sucrose (●), hexose (▲), and FS (■) of neutral fraction of pedicel (A) and endosperm (B) from [$^{14}C$]FS supplied to the husk leaf. Data are from one of three experiments with similar results.

uble fraction at 2.5 to 4 times the rate of accumulation of radiolabel from [$^{14}C$]FS during the first 8 h (Fig. 5). Between 8 and 11 h, accumulation of radiolabel into MCW-insolubles from supplied [$^{14}C$]sucrose plateaued, while the rate of accumulation from [$^{14}C$]FS increased to a rate on the order of the initial rate of accumulation from [$^{14}C$]sucrose (Fig. 5).

## DISCUSSION

The entry of FS into the endosperm suggests that inversion to hexoses is not necessary for movement of phloem-supplied sucrose from the maternal tissues into the endosperm. In fact, as others have pointed out (1, 7), we do not know what percentage of unloaded sucrose enters the endosperm as sucrose versus hexoses. Evidence from a variety of sources indicates that both hexoses and sucrose are present in the pedicel tissues. Equal molar amounts of hexose and sucrose in maize pedicel 19 d after pollination have been reported (9). In the present study, at steady state the ratio of $^{14}C$ in hexose to sucrose in the pedicel was 1.2 to 1.5, indicating that 40 to 45% of total imported radiolabel present in the pedicel was in sucrose. The presence of sucrose and hexoses in pedicel tissues, together with the fact that FS entered the endosperm without prior hydrolysis, is evidence that sucrose, in addition to hexoses, is taken up into the endosperm.

Using histochemical localization, Felker found invertase activity in maize basal endosperm and pedicel tissues, but not in the

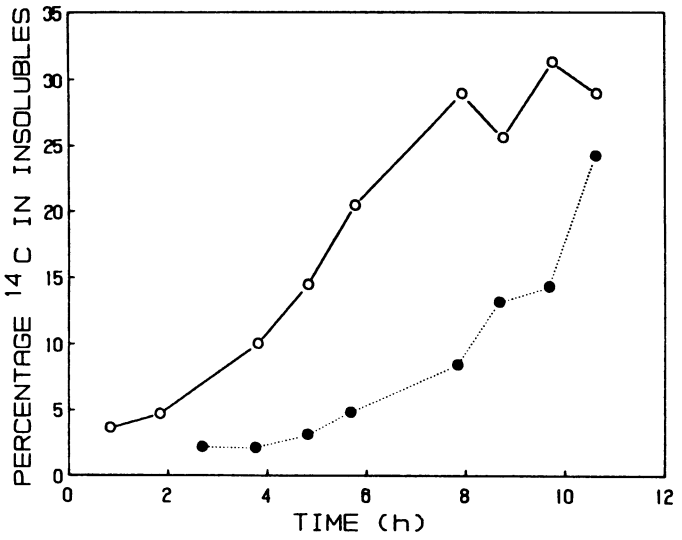


FIG. 5. Percent of total $^{14}C$ accumulated in endosperm in MCW-insoluble fraction from [$^{14}C$]sucrose (○) or [$^{14}C$]FS (●) supplied to the husk leaf. Data for each sugar are from one of three experiments with similar results.

intervening placental-chalazal tissue, suggesting that sucrose could be taken up into the endosperm and hydrolyzed there (1). The finding of invertase activity in basal endosperm is consistent with previously reported labeling patterns (10). When the ear leaf is given a pulse of $^{14}CO_2$, the highest percentage, 70%, of total $^{14}C$-label in hexose is found in the basal endosperm layer even after 4 h.

Recent results showing linear uptake of hexoses and sucrose into excised endosperm tissue at concentrations up to 100 to 200 mM, has led investigators to postulate passive uptake of glucose, fructose, and sucrose by basal endosperm (3, 5, 13). It is suggested that uptake into the endosperm follows a downhill concentration gradient from the pedicel region, which has a sugar concentration in the range of 470 to 800 mM (12). Our results are consistent with passive uptake into the endosperm of hexoses, sucrose, and FS when present.

Hexoses in the endosperm are used in the resynthesis of sucrose, which is used in synthesis of starch within the amyloplast (10). Many questions remain as to the enzymes involved in resynthesis and breakdown of sucrose in the endosperm and possible compartmentalization of the two processes. Results here confirm that sucrose in the endosperm is broken down by sucrose synthase. During the first 8 h, the ratio of sucrose entry into MCW-insolubles *versus* FS was 2.5 to 4, which is similar to the discrimination of 3.6 measured for sucrose *versus* FS in breakdown via sucrose synthase from immature soybean leaves and wheat germ (8).

The accumulation of [$^{14}C$]sucrose in the endosperm when [$^{14}C$]FS was supplied indicates that the glucose moiety of FS was used in sucrose synthesis (Fig. 4B). This implies that products of sucrose breakdown via sucrose synthase (in our case FS breakdown) were available as precursors for the resynthesis of sucrose. This availability is an important point when considering the compartmentalization of sucrose resynthesis and cleavage in the endosperm. Further, less than 5% of radiolabel entered the hexose pool from [$^{14}C$]FS even though [$^{14}C$]sucrose accounted for over 50% of the radiolabel in neutrals. Either [$^{14}C$]UDP-glucose, produced via sucrose synthase cleavage of [$^{14}C$]FS or [$^{14}C$]sucrose, was used directly for sucrose or starch synthesis, or the turnover rate of hexoses was too rapid to detect accumulation of radiolabel in hexoses. Synthesis of [$^{14}C$]sucrose from [$^{14}C$]FS followed by utilization in starch synthesis probably accounted for the increase in $^{14}C$-accumulation in the insoluble fraction after 8 h with [$^{14}C$]FS supply.

In conclusion, the entry of FS into the endosperm without hydrolysis raises the question of the role of invertase in basal maternal tissue. FS entry is evidence against the specificity of hexoses for uptake into the endosperm, and lends support to the postulated passive uptake of phloem sugars. FS metabolism in the endosperm emphasizes the importance of sucrose synthase in sucrose breakdown and demonstrates the availability of products of sucrose breakdown to the site of sucrose resynthesis, possibly via direct utilization of UDP-glucose. Further work to determine the enzymes involved in sucrose resynthesis and the compartmentalization of such enzymes and sucrose synthase are clearly needed.

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