Sugar Uptake and Metabolism in the Developing Endosperm of Tassel-seed Tunicate (Ts-5 Tu) Maize

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ABSTRACT

Factors regulating assimilate transport into developing maize (Zea mays L.) kernels have been difficult to determine because of the structural complexity of basal kernel tissues and the damage that results from tissue dissection. The sensitivity of maize kernels to experimental manipulation is such that substantial maternal tissue is required to support kernel growth in vitro. Consequently, sugar transport experiments with isolated seed tissues or detached kernels have not unequivocally demonstrated how sugar transport occurs. In the present study, Tassel-seed Tunicate (Ts-5 Tu) maize kernels were investigated as a model system for introducing test solutions into the pedicel apoplast with minimal wounding. Transpiration in leafy glumes drew 14C-sugar solutions up the 8- to 10-millimeter-long pedicel stalks into the basal endosperm transfer cell region. 12C from fructose was incorporated into starch for 8 days. Sugar uptake into endosperm and embryo tissue showed specificity and inhibitor sensitivity. In particular, p-chloromercuribenzenzene sulfonate partially inhibited fructose uptake into the endosperm but had no effect on the metabolic conversion of that fructose that entered the endosperm. These results are consistent with active, carrier-mediated sugar transport, but a definitive determination would require more detailed tissue analysis. We propose that further refinement of the incubation solution may allow long-term kernel growth without cob tissue and thus provide a more precise determination of which maternal factors influence seed development.

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The mechanisms regulating assimilate movement into developing maize (Zea mays L.) kernels are not known. Anatomical considerations dictate that assimilates must leave the symplast of the pedicel and enter the apoplast, from which they are absorbed by basal endosperm transfer cells (4). The physiological and biochemical processes that may mediate or limit assimilate transport remain obscured by the complexity and inaccessibility of the many tissues involved. Experiments in which the endosperm was replaced by an agar trap suggested that assimilates move passively into the pedicel apoplast (13). Excised basal endosperm transfer cell tissue was found to absorb sugars passively (15). Short-term sugar uptake into excised, developing kernels, from which most of the pedicel tissue was removed, appeared passive and insensitive to metabolic inhibitors (7). Similar studies by others (9), however, showed that glucose uptake kinetics were not strictly linear, suggesting that a carrier might be involved. The possibility of tissue damage caused by surgical manipulation or biochemical perturbation of the fragile tissues represents a significant difficulty in the interpretation of results obtained using such techniques. One indication of how sensitive maize kernels are to experimental manipulation is the requirement of a substantial amount of cob tissue to support kernel growth in vitro (3, and references therein).

This investigation of a potentially useful experimental system was aimed at minimizing tissue damage or wound effects while supplying test solutions to developing maize kernels. The unique morphological characteristics of the plant terminal inflorescence of Tassel-seed Tunicate (Ts-5 Tu) maize provides a useful model system for studying assimilate transport. The dominant mutations Ts-5 (Tassel-seed) and Tu (Tunicate, having leafy glumes covering the grain) were described by Nickerson and Dale (11). The Tunicate trait is believed to represent the ancestral origin of modern maize, being expressed in pod corn and other primitive strains (10). The glumes of Tunicate maize kernels resemble leaves more than glumes in structure and function (5). We have isolated a strain of maize expressing both Ts-5 and Tu characteristics. Many of the tassel kernels are born on a pedicel stalk (rachilla) 8 to 10 mm long. The excised, pedicellate, glume-covered kernels, produced in abundance (up to 100) on vigorous tassels, comprise up the experimental units in this study. This experimental system takes advantage of the transpiration-driven movement of incubating solution up the pedicel stalk and into the apoplast of the kernel base, near the point of glume attachment. Test solutions introduced to kernels in this way reach the apoplast of the basal endosperm transfer cell layer under conditions less likely to cause deleterious wound effects than would be possible with other systems.

MATERIALS AND METHODS

Plant Material

A line of Ts-5 maize (Zea mays L.) in a proprietary background that occasionally produced Ts-5 Tu plants was originally obtained from Dekalb Seed Co. in 1977 by Dr. J. C. Shannon (Pennsylvania State University). Through many generations of inbreeding and selection, a line of seeds expressing both Ts-5 and Tu was obtained. The last two generations were selected at the U.S. Department of Agriculture/Agricultural Research Service, National Center for Agricul-
Plants were grown in 12-L pots in Redi-earth Peat-lite soilless medium (W. R. Grace, Cambridge, MA) supplemented with Osmocote 14-14-14 and Micromax micronutrients (Sierra, Milpitas, CA). Plants received supplemental lighting for 16 h/d from sodium vapor and metal halide lamps and were hand pollinated.

**Fluorescent Dye Experiments**

Pedicellate Ts-5 Ts kernels were incubated in solutions of the apoplastic fluorescent tracer dye PTS\(^\text{3}^\) and sodium fluorescein, a symplastic tracer (both at 1% [w/v]) for 24 h. Movement of the dyes into the tissues was demonstrated by slicing the kernel and pedicel longitudinally from the top down and photographing in a darkroom with UV illumination.

**\(^{14}\)C-Sugar Uptake Experiments**

Kernels were excised 15 to 20 d after pollination and incubated in 1.5-mL microcentrifuge tubes containing 0.1 mL of medium at 25°C placed 40 cm from a 30-W fluorescent lamp. Incubation medium contained 4.3 g/L of Murashige and Skoog salts, 2 mg/mL of streptomycin, 45 mM glutamine, and the \(^{14}\)C-sugar (0.1 \(\mu\)Ci/mL) at the concentration indicated for each experiment. Uptake of \(^{14}\)C was determined by oxidizing lyophilized tissue with a Packard sample oxidizer. At least three replications of five kernels each were used per treatment unless indicated otherwise. To determine incorporation of \(^{14}\)C into starch, endosperms were homogenized with a Polytron and extracted with boiling 80% ethanol, centrifuged, and rinsed three times with 80% ethanol; the residue was dried and oxidized. To determine the distribution of \(^{14}\)C among sugars, soluble polysaccharides, and starch in the endosperms, they were ground in 80% ethanol with a mortar and pestle, centrifuged, and rinsed as above, and aliquots were analyzed by TLC on 0.25-mm silica gel plates. The mobile phase was methyl ethyl ketone:ethanol:water (90:10:8.7), and chromatograms were developed five times. \(^{14}\)C distribution among fructose, glucose, sucrose, and an 80% ethanol-soluble fraction with less chromatographic mobility than sucrose (presumably oligo- or polysaccharides) was determined with a radioanalytical imaging system (AM-BIS Systems, Inc., San Diego, CA).

**RESULTS AND DISCUSSION**

**Morphology of Kernel Units**

The terminal inflorescence of a Ts-5 Ts plant 20 d after pollination is shown in Figure 1a. Arrangement of single and paired kernel units along a tassel branch (Fig. 1b) is similar to staminate florets of normal maize. In tassels of the desired phenotype, kernel units are born on pedicel stalks 8 to 10 mm long (Fig. 1c). Removal of one of the leafy glumes and a variable number of other floral structures (palea, lemma, and additional glumes) reveals a typical maize kernel, usually sessile to the point of glume attachment (Fig. 1d). Single, pedicellate kernel units alternate with sessile kernel pairs, but a vigorous plant may bear more than 100 pedicellate kernel units. The tips of some branches bear staminate flowers, which provides a means of self-pollinating a tassel by enclosing it in a bag so as to prevent the usual recurvature (Fig. 1a).

**Fluorescent Dye Movement**

PTS is a small, negatively charged dye that has been used to demonstrate apoplastic pathways in plant tissues (12). Fluorescein enters plant tissue both apoplastically and symplastically (1). Both PTS and fluorescein permeated the glume tissue within 24 h (Fig. 2, a and c). Whereas PTS was visible only in the pedicel tissue of the kernel (Fig. 2b), fluorescein was evident throughout the endosperm and embryo also (Fig. 2d). Either diffusion or transpiration occurring in the leafy glumes drew incubating solution up through the xylem into the apoplast of the kernel base, from where fluorescein but not PTS passed through the plasmalemma of the basal endosperm transfer cells into the endosperm symplast.

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\(^{3}\) Abbreviations: PTS, 3-hydroxy-5,8,10-pyrenetrisulfonate; DNP, dinitrophenol; PCMBS, p-chloromercuribenzene sulfonate; ESP, ethanol-soluble polysaccharide.
Figure 3. Time course of uptake of $^{14}$C from 100 mM $[^{14}$C]fructose into the endosperm, embryo, and pedicel/glumes of Ts-5 Tu kernels incubated for 24 h. Data are means of three replications of five kernels each. Bars represent ± se.

Figure 4. Accumulation of $^{14}$C in 80% ethanol-soluble and insoluble fractions of endosperms of Ts-5 Tu kernels incubated in 100 mM $[^{14}$C]fructose for up to 8 d. The incubation solution was exchanged with fresh medium daily. Data are means of three replications of five kernels each. Bars represent ± se.

though the kernel units under these experimental conditions were able to incorporate $^{14}$C into starch for 8 d, no significant increase in endosperm dry weight could be detected (data not shown). We assume that some maternal factor necessary for normal kernel metabolism was not provided by the incubation medium or the pedicel/glume tissue. In contrast, cob-born kernels cultured in vitro exhibit growth comparable to intact kernels (2), but the complexity of that system and the participation of cob tissue in sugar uptake do not allow experimental control of the pedicel apoplast composition (3).

The contribution of transpiration to the ascension of incubating solution up the pedicel was demonstrated by comparing uptake of $^{14}$C from $[^{14}$C]fructose into endosperm and pedicel/glume tissue in the light versus darkness. Significantly greater uptake into both tissues occurred in the light (Fig. 5). Because there was no temperature difference between light- and dark-incubated kernels, it is possible that light-driven transpiration from the stomates of the glumes contributed to

**[^14C]Fructose Uptake**

Radioactivity from $[^{14}$C]fructose accumulated first in the pedicel and glume tissue, and after a 3-h lag period, $^{14}$C was detected in the endosperm and embryo (Fig. 3). Between 12 and 24 h, the rate of increase in radioactivity on a dry weight basis was much greater in the endosperm than in the pedicel/glumes, consistent with the expectation that the endosperm would have a higher sink strength than the other tissues. When the kernels were incubated in $[^{14}$C]fructose for 8 d, radioactivity in the insoluble fraction of the endosperm increased steadily, whereas after 2 d the amount of radioactivity in the soluble endosperm fraction leveled off (Fig. 4).
Figure 5. Effect of light on uptake of $^{14}$C into the endosperm and pedicel/glumes of Ts-5 Tu kernels incubated for 8 h in 100 mM $[^{14}$C]fructose. Kernels were incubated 20 cm from a fluorescent lamp bearing two Sylvania Cool White 15-W bulbs, with aluminum foil arranged to restrict light but not air flow to the dark-treatment kernels. There was no temperature difference between the treatments. Data are means of 10 kernels per treatment. Bars represent ± SE.

Figure 6. Sugar uptake into the pedicel/glumes (A), embryos (B), and endosperms (C) of Ts-5 Tu kernels incubated for 6 h in 100 mM $[^{14}$C]sucrose (suc), $[^{14}$C]glucose (D-gluc), $[^{14}$C]fructose (fru), or $[^{14}$C]l-glucose (l-gluc). Uptake is expressed as millimole equivalents of the fed sugar per five tissue pieces (mean ± se of three replications).

Figure 7. Fructose uptake by pedicel/glumes (A), embryos (B), and endosperms (C) of Ts-5 Tu kernels incubated for 6 h in 100 mM $[^{14}$C]fructose containing 5 mM PCMBS, NaN$_3$, DNP, or Na$_3$VO$_4$. Uptake is expressed as a percentage of the control treatment (no inhibitor) and represents the mean ± se of five tissue pieces (three replications).

The enhancement of movement into the pedicel. Increased uptake into the endosperm may have been a result of more $^{14}$C being available in the pedicel apoplast. Alternatively, other light effects such as photosynthetic oxygen generation might have contributed.

Sugar Specificity and Inhibitor Effects

Although the relative amounts of $^{14}$C from sucrose, d-glucose, and fructose entering pedicel/glume tissue did not differ, significantly less l-glucose than d-glucose was absorbed (Fig. 6A). Because l-glucose is not actively transported into or metabolized in higher plant cells, uptake of l-glucose into a tissue serves as an estimate of the apoplastic component (8). Embryos showed preferential accumulation of d-glucose, and the difference between d- and l-glucose was more pronounced than in the pedicel/glumes (Fig. 6B). Endosperms showed little specificity among the metabolic sugars but, like the embryos, absorbed relatively little l-glucose (Fig. 6C). The differences observed between the incorporation of the metabolizable sugars may reflect differences in the rates of their metabolic conversion during the 6-h incubation time.

The sensitivity of fructose uptake to metabolic and transport inhibitors was examined. Neither PCMBS, NaN$_3$, DNP, nor Na$_3$VO$_4$ significantly inhibited accumulation of $^{14}$C from fructose in the pedicel/glumes (Fig. 7A). However, uptake
into embryos and endosperms was inhibited about 50% (overall) by these compounds. PCMBS is considered a non-penetrating inhibitor (6), whereas the NaN₃ and DNP readily enter the symplast and would be expected to inhibit aerobic metabolism generally. Vanadate is an ATPase inhibitor and would affect active transport into the basal endosperm transfer cells if such transport existed. By itself, this experiment does not allow us to distinguish between the inhibition of transport versus metabolism, but insofar as PCMBS is considered to be restricted to the apoplast, the possibility remains that transport by plasmalemma-bound carriers was affected. This point was specifically addressed in the following experiments.

**Metabolism of Transported Fructose**

Kernel units incubated in [¹⁴C]fructose were harvested at short intervals up to 2 h, and the distribution of [¹⁴C] among soluble sugars, ESPs, and starch was determined (Fig. 8). As expected, the [¹⁴C] in the initial sample was mostly in fructose, but metabolic conversion of fructose to sucrose, glucose, and the ESP fraction was rapid. By 100 min, the relative amount of [¹⁴C] in sucrose exceeded that in fructose, the [¹⁴C]-sugar supplied. The decrease to about 35% in the fraction of [¹⁴C] as fructose while the other pools increased suggests that the cytoplasmic pool of fructose is relatively small. Apparently the turnover of carbon from fructose was so rapid that [¹⁴C]fructose did not build up in the endosperm as transport progressed. However, the metabolic fate of [¹⁴C]fructose in endosperms incubated for 2 h in [¹⁴C]fructose with or without PCMBS was nearly identical (Fig. 9), even though the total [¹⁴C] recovered from the PCMBS-incubated endosperms was 67% of that from control endosperms. The similarity in the percentage of [¹⁴C] recovered as fructose suggests that PCMBS did not enter the endosperm and disrupt metabolism but, instead, affected transport of fructose into the endosperm at the level of the basal endosperm transfer cells.

Figure 8. Distribution of [¹⁴C] among fructose, sucrose, glucose, ESP, and starch (ethanol insoluble) fractions of endosperms of Ts-5 Tu kernels incubated for 20 to 120 min in [¹⁴C]fructose. To maximize the specific activity of fructose entering the endosperm, no carrier fructose was added ([fructose] < 1 mM). Data represent mean ± se of six endosperms.

Figure 9. Effect of 5 mM PCMBS added to the incubation solution on the distribution of [¹⁴C] among fructose, sucrose, glucose, and ESP fractions of endosperms of Ts-5 Tu kernels incubated for 120 min in carrier free [¹⁴C]fructose. Data represent mean ± se of six endosperms.

PCMBS could affect sugar transport into maize endosperm by inhibiting carrier-mediated transport across the plasmalemma of the basal endosperm transfer cells or by inhibiting invertase activity in the pedicel apoplast (13). Although [¹⁴C]fructose was used for this experiment to eliminate the latter effect, a higher percentage of [¹⁴C] recovered as sucrose in the PCMBS-treated endosperms (Fig. 9) may reflect a PCMBS inhibition of invertase-mediated cleavage of sucrose synthesized from fructose in the pedicel tissue. Singh et al. (14) observed PCMBS inhibition of [¹⁴C]sucrose transport into sorghum caryopses but also could not distinguish between apoplastic invertase inhibition and a direct effect on sugar transport as possible mechanisms of inhibition.

**CONCLUSIONS**

These experiments establish that Ts-5 Tu maize kernels can be used to introduce experimental solutions to the pedicel apoplast in the vicinity of the basal endosperm transfer cells without the wounding associated with detachment of kernels from normal maize ears. Pedicellate Ts-5 Tu kernels continued to synthesize starch from [¹⁴C]fructose for at least 8 d in culture. Further refinements of the incubation solution or conditions could result in long-term culture and growth of Ts-5 Tu kernels, circumventing the requirement of cob tissue participation in *in vitro* maize kernel culture. When such refinements are made, it will be possible to use the experimental system to determine more precisely what maternal factors contribute to kernel development.

The nonpermeant sulfhydryl reagent PCMBS partially inhibited transport of [¹⁴C]fructose into the endosperm but did not affect the metabolic conversion of fructose to other compounds in the endosperm. Uptake of exogenous sugars into the endosperm and embryo showed sugar specificity and inhibitor sensitivity. Although these results are consistent with active carrier-mediated transport of sugars, more detailed temporal and spatial analysis of kernel tissue would be necessary for a definitive determination. The Ts-5 Tu kernel incubation procedure described here provides an appropriate system for such experiments.
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LITERATURE CITED