Plant Physiol. (1982) 70, 322–325
0032-0889/82/70/0322/04/$00.50/0

Short Communication

Source-Sink Relations in Maize Mutants with Starch-Deficient Endosperms[1]

Received for publication November 9, 1981 and in revised form March 17, 1982

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ABSTRACT

Partitioning and translocation of photosynthates were compared between a nonmutant genotype (Oh 43) of corn (*Zea mays* L.) and two starch-deficient endosperm mutants, *shrunken-2* (*sh2*) and *brittle-1* (*bt1*), with similar genetic backgrounds. Steady-state levels of $^{14}CO_2$ were supplied to source leaf blades for 2-hour periods, followed by separation and identification of ^{14}C-assimilates in the leaf, kernel, and along the translocation path. An average of 14.1% of the total ^{14}C assimilated was translocated to normal kernels, *versus* 0.9% in *sh2* kernels and 2.6% in *bt1* kernels. Over 98% of the kernel ^{14}C was in free sugars, and further analysis of nonmutant kernels showed 46% of this label in glucose and fructose. Source leaves of mutant plants exported significantly less total photosynthate (24.0% and 36.3% in *sh2* and *bt1* compared to 48.0% in the normal plants) and accumulated greater portions of label in the insoluble (starch) fraction. Mutant plants also showed lower percentages of photosynthate in the leaf blade and sheath below the exposed blade area. The starch-deficient endosperm mutants influence the partitioning and translocation of photosynthates and provide a valuable tool for the study of source-sink relations.

A high economic yield of grain crops depends on efficient translocation of assimilates to kernel sink tissues. In the present study, maize mutants with reduced kernel capacity for starch accumulation have been used to examine the effects of sink strength on partitioning of assimilates in source leaves and their subsequent distribution within the plant. There are several advantages to using plants with mutations affecting sink metabolism for such a study. First, mechanical manipulation of source leaves or sink tissues can be avoided. Such manipulations might result in short-term decreases in phloem functioning (6) or atypical changes in the hormonal balance within the plant (9). Secondly, inhibitors are not used, avoiding other possible secondary effects (25).

Two maize mutants, *brittle-1* (*bt1*) and *shrunken-2* (*sh2*), synthesize about 25% of the starch of nonmutant endosperms and consequently have shrunken endosperms at maturity (15). The biochemical lesion resulting from the *bt1* mutation has not been identified, but it is known that the *sh2* mutation results in a pronounced reduction in ADP-glucose pyrophosphorylase (EC 2.7.7.b) activity (2, 24). The *sh2* mutation affects only the endosperm ADP-glucose pyrophosphorylase activity (17) and does not affect that activity in the embryo, or sporophytic generation. For this reason, the differences in photosynthate partitioning and translocation reported in the present study for *sh2* plants would result from the reduced starch synthesis in the endosperm, rather than a genetic change in the source leaf. At this time, we cannot make the same statement concerning the *brittle-1* mutation, although preliminary studies have indicated photosynthate partitioning is similar in these two mutants.

MATERIALS AND METHODS

PLANT MATERIAL. Three corn genotypes were examined in this study; 'nonmutant' inbred (Oh 43) of maize (*Zea mays* L.), and two starch-deficient mutants, *shrunken-2* and *brittle-1* in the same genetic background. We will refer to homozygous mutant *sh2/sh2* plants as *sh2* plants and to homozygous mutant seeds (*sh2/sh2* embryos with associated *sh2/sh2/sh2* endosperms) as *sh2* seeds. The same type of abbreviation is used for *brittle-1*. Plants were grown in the field using 12-liter buried metal containers, watered as needed during the growing season, and self-pollinated. Development proceeded at rates similar to other field-grown inbreds. Experimental plants were transported to the laboratory for studies done between 20 and 23 d after pollination.

$^{14}CO_2$ Labeling. Two-h, steady-state labeling studies were done by supplying $^{14}CO_2$ to a source leaf blade of each plant. The ear leaf blade was used for these feedings whenever fully intact; otherwise the leaf one internode above was used. This difference resulted in minimal changes of overall partitioning or translocation (discussed later). One h before each labeling period, a central 30-cm portion of the leaf blade was sealed in a 350-ml chamber with circulating air and allowed to equilibrate. Photosynthetic photon flux density was 550 to 750 $\mu E\ cm^{-2}\ s^{-1}$, and the temperature was maintained at 31°C at the leaf surface. The combined level of $^{14}CO_2$ and $^{12}CO_2$ was kept between 250 and 350 $\mu l/l$ throughout each labeling period using a closed system with an IR gas analyzer and a timed injection syringe (10). The amount of $^{14}CO_2$ assimilated by each plant over a 2-h period varied between 67 and 152 μCi because of differences in leaf size and photosynthetic rate.

Photosynthetic rates were measured before and during each labeling period by measuring disappearance of CO_2. Rates were not correlated with time of day that $^{14}CO_2$ fixation took place (data not shown). Also, preliminary 12-h measurements of both mutant and nonmutant genotypes showed only slight variations between 0800 and 1800 h (the period during which labeling studies were conducted later).

Plant tissues were separated immediately after each labeling period and frozen in liquid N_2. Tissue fractions were as follows: exposed area of the source leaf blade, blade below the fed area,

[1] Supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, and by National Sciences Foundation Grant PCM-7712884.

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sheath, node at the source leaf insertion, shank, husks, cob, kernels, and internodes above and below the fed leaf. In those instances where the leaf above the ear-leaf was used, the stem internode between this leaf and the ear was also sampled. Kernels, including the chalazal zone, were detached from cobs while frozen. Developed and aborted kernels were separated, and little or no detectable radioactivity was found in the latter. Roots and upper plant parts were not sampled, but the amount of label in these remaining tissues was estimated by subtracting the amount of radioactivity recovered from the other plant parts from the total $^{14}CO_2$ assimilated.

Samples were boiled in 80% (v/v) ethanol to extract soluble assimilates. Boiled tissues were finely chopped and homogenized in an Omni-mixer. Residue was washed two to four times with 80% ethanol, and twice more with cold water. The amount of ^{14}C in the residue was determined by placing weighed portions in vials, adding liquid scintillation fluid (5.5 g/l PPO in toluene:methyl cellusolve, 2:1), and counting radioactivity. A quench curve for Chl was used with the external standard to convert cpm to dpm. Aliquots of the soluble assimilate fraction obtained from the pooled washing were used to determine the total ^{14}C content for each extract.

For kernels, the assimilates were separated into insolubles (predominantly starch), a chloroform extractable fraction (oils), water-soluble polysaccharides, protein, and free sugars as described by Shannon (19). Specific activities of free-sugar fractions from nonmutant kernels were high enough to allow further analysis. Sucrose, glucose, and fructose were separated via paper chromatography using butanol, ethanol, and water (13:8:4) which is a modification of Shannon's method (19).

Calculations of specific activities of sucrose along the translocation path were based on a modified resorcinol assay for sucrose (1) and radioactivity determinations.

RESULTS AND DISCUSSION

Source-sink relationships are altered in the corn mutants with starch-deficient endosperms. The influence of these alterations on photosynthesis in the leaf blade subtending the ear (ear leaf blade) was investigated. Mean photosynthetic rates for the ear leaf blades (expressed as mg CO_2 dm^{-2} h^{-1}) of the nonmutant and mutant plants were as follows: nonmutants = 38.3 ± 11.5; *sh2* = 29.4 ± 0.8; *btl* = 24.0 ± 4.1. Because of the small number of plants sampled and the large SE for the nonmutants, the differences were not statistically significant. However, the data show an interesting trend, suggesting that the reduced sink capacity of the mutants may result in lower photosynthetic rates in the source leaves of those plants.

The overall patterns of ^{14}C-assimilate distribution showed significant differences between nonmutant and mutant genotypes (Table 1). Considerably more ^{14}C-photosynthate was retained in the exposed area of *sh2* and *btl* leaves (76–65%, respectively) compared to nonmutant leaves (52%). Corn is an efficient exporter of photosynthate, particularly during the reproductive phase (23), but the higher levels of labeled assimilates remaining in leaves of the mutant plants were more comparable to those observed in soybean (12). Among nonmutant and *btl* plants, slightly more photosynthate accumulated in the source leaves when those above the ear were used. This might be expected on the basis of differing proximity to the sink (23), but the variation did not adversely influence the analysis of differences in assimilate export among the genotypes.

A striking difference between nonmutant and *sh2* mutant genotypes was evident in the amount of photosynthate translocated to the kernels with 14.1% of the total ^{14}C in the nonmutant kernels compared to 0.9% in the *sh2* kernels (Table I). A similar trend was observed in the *btl* plants. These results are consistent with the contrast in sink-demand for carbon between the groups. They are not necessarily proportional to the levels of starch in the endosperm, however, because nearly all of the kernel radioactivity was found in ^{14}C-sugars (discussed later). Shannon (18) also found this to be the case, even 6 h after a $^{14}CO_2$ pulse feeding.

The *sh2* plants also showed significantly reduced percentages of ^{14}C-photosynthate in cobs ($p < 0.05$) and reduced translocation into the whole sink area. This might be expected on the basis of carbon utilization in kernels because the cob alone would not show a high demand for assimilates. There is no build-up of ^{14}C near the terminal end of the translocation path as might occur, according to Jenner (11), if there were a barrier to assimilate entrance into the kernel. Accumulation of ^{14}C-photosynthate in the leaves of both mutant genotypes may possibly indicate some form of regulation in the source tissue of these plants.

There are several proposed regulatory points within a source leaf that may be influenced by a kernel mutation. First, the rate of sucrose and starch formation in the source leaf may be regulated either directly or indirectly by the sink tissue. With a decreased demand for carbon, greater quantities of sucrose and triosephosphate could accumulate in the mesophyll cytoplasm, thus inhibiting triosephosphate export from the chloroplast and stimulating starch formation (8, 25). Starch build-up has also been correlated with decreased CO_2 fixation rates in some studies (8, 25). Second, efflux of transport compounds into the apoplast prior to phloem loading may be involved in the control of translocation out of source leaves (5). Efflux could be affected by the rate of sucrose synthesis and release from the chloroplast (7) and also by vacuolar compartmentalization (16). In addition, the phloem-loading process may be influenced by altered source-sink relations perhaps in response to changes in phloem turgor (21) through a solute flow mechanism proposed by Milburn (13). Last, it is also possible that observed changes in source-leaf metabolism may in part be brought about through hormonal effects (9).

Levels of ^{14}C in husks and stem internode above the fed leaf

Table I. *Distribution of ^{14}C between Plant Tissues of Three Zea mays L. Genotypes after Source-Leaf Exposure to 2 Hours of Steady-State $^{14}CO_2$ Levels*

Values for n = 4, 3, and 2, respectively, for nonmutant, *shrunken-2*, and *brittle-1*.

Genotype	Fed Source Leaf	Blade Below + Sheath	Node or Stem Between	Shank	Cob	Kernels	Husks	Stem Internode Above	Stem Internode Below	Unsampled Tissues[a]
	% recovered of total dpm fed ± SE									
Nonmutant	52.0 ± 4.6	10.9 ± 1.2	2.2 ± 1.9	3.2 ± 0.6	3.4 ± 1.0	14.1 ± 6.0	0.3 ± 0.1	0.8 ± 0.8	3.4 ± 1.3	9.8 ± 6.6
Shrunken-2	76.0 ± 2.7[b]	6.5 ± 1.2[b]	4.4 ± 3.1	3.1 ± 2.0	0.7 ± 0.5[c]	0.9 ± 0.2[c]	0.3 ± 0.5	0.1 ± 0.1	3.5 ± 2.7	4.5 ± 2.1
Brittle-1	65.1 ± 3.1[c]	5.1 ± 0.6[b]	0.6 ± 0.4	2.1 ± 0.6	1.7 ± 0.4	2.6 ± 2.0	0.1 ± 0.1	0.1 ± 0.1	5.6 ± 0.4	17.3 ± 4.5

[a] Predominantly roots and upper plant parts (also includes any unspecified losses).

[b] $p < 0.01$; fractions significantly different from those of nonmutant plants based on a t test.

[c] $p < 0.05$; fractions significantly different from those of nonmutant plants based on a t test.

Table II. *Distribution of ^{14}C between Assimilates in Kernels of Three Zea mays L. Genotypes after Source-Leaf Exposure to 2 Hours of Steady-State $^{14}CO_2$ Levels*

Values for n = 4, 3, and 2, respectively, for nonmutant, *shrunken-2*, and *brittle-1* plants.

Genotype	Insoluble Assimilates (starch)	Chloroform Fraction (oil)	Water-Soluble Polysaccharides	Protein	Free Sugars
			% total kernel dpm ± SE		
Nonmutant	0.730 ± 0.281	0.149 ± 0.057	0.093 ± 0.117	0.175 ± 0.189	98.854 ± 0.575
Shrunken-2	0.115 ± 0.163[a]	1.200 ± .141[b]	0.094 ± 0.233	0.162 ± 0.229	98.429 ± 0.4
Brittle-1	0.155 ± 0.064[a]	3.300 ± 3.253	0.137 ± 0.047	0.126 ± .127	96.280 ± 3.5

[a] $p < 0.05$; fractions significantly different from those of nonmutant plants based on a t test.
[b] $p < 0.01$; fractions significantly different from those of nonmutant plants based on a t test.

Table III. *Distribution of ^{14}C between Soluble and Insoluble Assimilates (Predominantly Starch) in Source Leaves of Three Zea mays L. Genotypes after Source-Leaf Exposure to 2 Hours of Steady-State $^{14}CO_2$ Levels*

Values for ;2n;1 = 4, 3, and 2, respectively.

Genotype	^{14}C-Assimilates in Source Leaves	
	Soluble fraction	Insoluble fraction
	% ± SE	
Nonmutant	95.3 ± 1.0	4.7 ± 1.0
Shrunken-2	84.9 ± 6.0[a]	15.1 ± 6.0[a]
Brittle-1	87.9 ± 8.7	12.2 ± 8.7

[a] $p < 0.01$; means significantly different from those of nonmutant plants based on a t test.

averaged less than 1% of the total (Table I). Thus, neither was a significant sink for assimilates. Slightly more ^{14}C-photosynthate moved upward in nonmutant plants when the leaf above the ear was used, but as in earlier studies (23), basipetal translocation predominated after tasseling. Few differences were apparent in percentages of radioactivity in lower stems. Although an estimated value, 'roots and upper plant parts' probably represents mainly root ^{14}C because of the translocation patterns mentioned above.

Less than 1% of the label in the kernels was found in insoluble compounds (Table II), but appreciably more labeled starch occurred in kernels of nonmutant plants. In *sh2* mutant kernels, however, significantly more ^{14}C was found in the oil (chloroform fraction) with 1.2% compared to only 0.1% in the nonmutant kernels. The higher percentage of dpm in the oil fraction of the *sh2* mutant may suggest that assimilates are diverted to the germ.

Composition of the free ^{14}C-sugars in the kernels was examined further to determine the degree of sucrose hydrolysis which occurred. Specific activities of these assimilates were high enough for accurate separation and identification only in nonmutant kernels. Only 54% of the free-sugar ^{14}C occurred in sucrose, whereas the remaining radioactivity was in glucose and fructose (data not shown). These results are consistent with Shannon's report (19) of sucrose hydrolysis on entry into corn endosperm. The sucrose/reducing sugar ratios of labeled compounds in the present study are also similar to those found in the earlier studies (19).

The percentage of ^{14}C label in starch (the predominant portion of the insoluble fraction) in the source leaves varied with genotype (Table III). In the nonmutant, only 4.7% of the total leaf ^{14}C was in the starch fraction, whereas 15.1% and 12.2% was in starch in leaves of *sh2* and *btl* mutants, respectively. These differences in starch synthesis at the source are consistent with other reports on the effects of different source/sink ratios (3, 14). This accumulation also agrees with reports of starch build-up and reduced photosynthetic rate occurring during decreased translocation from the leaf (5, 22). Conversely, the low rate of starch accumulation and effective translocation out of source leaves of nonmutant plants has been attributed to the proximity of an active sink such as a developing cob (23).

The specific activity of sucrose along the translocation path in the maize genotypes was measured to determine the possibility of reduced photosynthate export from mutant leaves due to build-up of unlabeled sucrose in the phloem. Several authors have suggested the importance of a decreasing concentration gradient between source and sink for effective translocation (8, 25). In this study, unlabeled sucrose did not appear to accumulate any more in the paths of mutant than nonmutant plants (data not shown).

Amino acids in the sheaths were examined and found to comprise less than 3% of the total radioactivity. This is substantially less than the levels found in the translocation path of soybeans (10, 12), but might be expected since the protein concentration and hence the nitrogen demand of corn is much lower than in soybeans (20).

The sink, source, or path may influence the translocation of photosynthates in plants under various conditions (5). This control can be both direct and indirect and may involve an interaction of long-term hormonal influences (4, 8, 22) with the more immediate effects of concentration gradients and regulatory sites along the translocation path (8, 22). In the present study, the mutation *sh2* (and possibly *btl*) affects only the endosperm sink tissue; yet partitioning and translocation of photosynthates apparently reflect changes in the source leaves. Several regulatory sites in source leaves could have been indirectly affected by the changes in the kernels, such as starch and sucrose synthesis in the mesophyll or bundle sheath cells, efflux of photosynthates into the apoplast, or phloem loading. The *sh2* and *btl* mutants as well as other mutants with markedly reduced starch synthesis should provide useful probes for further investigations in this area.

Acknowledgment—The authors thank Mark Saliba for valuable technical assistance with these experiments.

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