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Phloem Loading in Squash

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ABSTRACT

Squash (*Cucurbita pepo* L. var. *melopepo torticalis*, Bailey) leaves were supplied with ^{14}C-sucrose, then specific radioactivities of the glucose and galactose moieties of translocated stachyose were determined. In every case, the specific radioactivity of the galactose moiety was greater than that of the glucose moiety. It is concluded that the stachyose was not synthesized at either the phloem-loading site or subsequent to phloem loading, but rather in cells that were not a part of the translocation system, possibly the mesophyll cells.

Loading of carbohydrate into minor veins of leaves is the process that controls both the qualitative and quantitative components of translocation. Until recently there has been little direct evidence regarding the pathway followed by carbohydrates as they move from the photosynthetic cells into the phloem. Recently, however, Geiger *et al.* (5) and Giaquinta (6) have presented strong evidence that this pathway involves the apoplast. This paper presents indirect evidence in support of this apoplastic theory of phloem loading; however, the main thrust of this paper relates to the elucidation of the chemical specificity of sites which load carbohydrates into the phloem from the apoplast.

A number of Soviet workers (2, 3, 9, 10) concluded that the phloem-loading system is specific to hexoses. From this, one would presume that the translocated oligosaccharides are synthesized either during or subsequent to loading. Geiger *et al.* (5) have presented evidence that the loading site in sugar beet leaves is specific to sucrose.

In this study ^{14}C-sucrose was supplied to leaf blades of squash, a species which normally translocates primarily stachyose. After translocation had occurred, the labeling pattern of the galactose and glucose moieties of stachyose from the petioles was determined. These results, together with results from earlier work (5-8) are used to support the conclusion that the loading site is specific to the translocated carbohydrate.

MATERIALS AND METHODS

Except for the hydrolysis of the stachyose and the analysis of the resultant hexoses, the procedures used were essentially the same as have been reported previously (8). Therefore, only a brief description is presented here.

Plant Material. *Cucurbita pepo* L. var. *melopepo torticalis*, Bailey seeds were germinated in vermiculite, and the resultant seedlings were transferred to modified Meyer's solution (7) for growth in the greenhouse. When the second true leaf was fully expanded, the plants were transferred to a fume hood. Light from 300 w reflector flood incandescent bulbs was filtered through water, supplying 1,000 ft-c to the plants. The day before label was supplied to the plants, all leaves were removed except the youngest fully expanded leaf (labeled leaf) and leaves less than one-fifth fully expanded.

Experimental Procedure. Uniformly labeled ^{14}C-sucrose (5.2 mCi/mmol) was introduced through the second lateral vein from the leaf base by the reverse flap method of Biddulph (1). After an appropriate labeling period, the petiole of the labeled leaf was cut into sections and each section placed in hot, 95% v/v ethanol.

Extraction, Purification, and Analysis. Each petiole segment was extracted with 80% v/v ethanol in a Microsoxhlet for 24 hr. The constituents of the extracts were separated by descending chromatography on Whatman No. 1 paper using 5:3:2 v/v/v 1-butanol-ethanol-water solvent. Known sugars were chromatographed on the same sheets adjacent to the samples. Each chromatogram was autoradiographed and the radioactive spot corresponding to known stachyose was cut out and eluted.

Each eluted sample was dried and the residual stachyose was hydrolyzed in a solution of α-galactosidase prepared according to English *et al.* (4) containing 1 mg/ml protein in 0.1 M Na-acetate buffer (pH 5.2). One-half ml of this solution was added to each stachyose sample and incubated for 4 hr at 27 C; then the samples were dried.

The hydrolyzed samples were chromatographed for 108 hr using the organic phase of 9:1:10 v/v/v 1-butanol-ethanol-water. These chromatographs were autoradiographed and each radioactive area cut out and ^{14}C present was measured by liquid scintillation counting. It is apparent that there was invertase activity in the protein, for considerable ^{14}C appeared adjacent to the glucose and fructose standards, as well as adjacent to galactose. The scintillation compounds were rinsed from the paper, then the sugars were eluted. Galactose and glucose were quantitatively determined using galactose oxidase and glucose oxidase from Worthington Biochemical Corp., Freehold, N.J., as previously described (7).

RESULTS

The specific radioactivities of glucose and galactose derived from translocated stachyose are plotted against distance from the source blade in Figures 1 and 2. The points are plotted to indicate the center of each 4-cm petiole piece. As can be noted, the specific radioactivity of the derived galactose was greater, in every case, than that of the derived glucose. Since the ^{14}C which traveled the greatest distance through the petiole was exported from the leaf at the earliest time, the distance along the petiole represents a time sequence of biochemical events within the blade.

One might expect that the amount of ^{14}C from the petiole of the leaf labeled for 40 min should be greater than from the one treated for 30 min. However, there is considerable variation from plant to plant in the rate that the solution enters through the cut vein.

A plant was labeled for 60 min; in that case, the specific radioactivities of the two hexoses were not as divergent because the system was approaching isotopic equilibrium. However, even after 60 min of labeling, the specific radioactivity of the galactose moiety of stachyose was greater than that of the glucose moiety in every petiole piece.

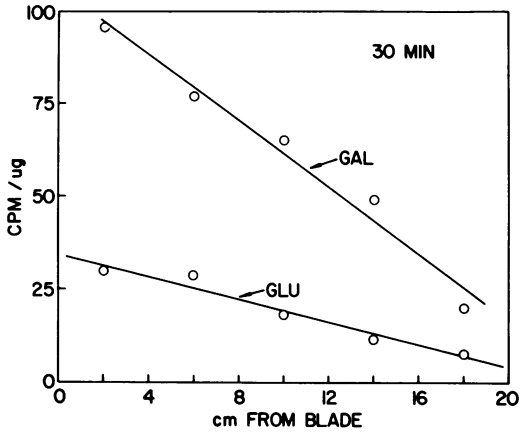

FIG. 1. Specific radioactivities of glucose and galactose moieties of stachyose extracted from petiole sections. Distance indicated represents the center of each 4-cm petiole section. Blade labeled for 30 min with ^{14}C-sucrose.

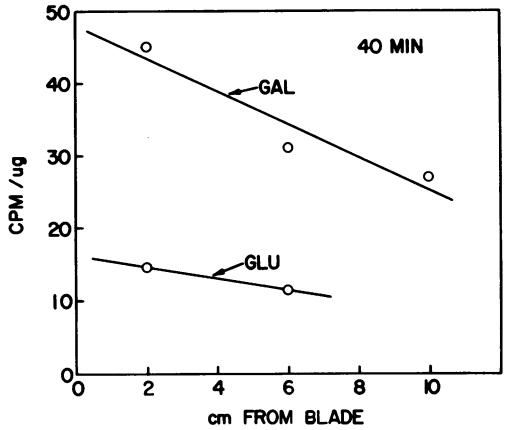

FIG. 2. Specific radioactivities of glucose and galactose moieties of stachyose extracted from petiole sections. Distance indicated represents the center of each 4-cm petiole section. Blade labeled for 40 min with ^{14}C-sucrose. Only three points are indicated for galactose and two for glucose since no detectable darkening occurred on the autoradiographic films of chromatographed samples taken at a greater distance from the source leaf.

DISCUSSION

When $^{14}CO_2$ was used to label the translocation stream of squash plants, most of the label appeared in stachyose (7). However, when ^{14}C-sucrose was used, most of the translocated label was in sucrose (8), but with time an increasing proportion of the label appeared in stachyose. The conclusion from these two reports was that stachyose is the carbohydrate normally translocated by squash plants, but that sucrose can be loaded into the phloem if it is supplied to the phloem-loading sites. These data suggest that the normally translocated material, stachyose, is not synthesized as it is loaded; for if the loading sites were specific to hexoses and the translocated oligosaccharide(s) were synthesized as they were loaded or subsequent to loading (2, 3, 9, 10), then the distribution of ^{14}C between sucrose and stachyose should not be dependent on the chemical form supplied ($^{14}CO_2$ or ^{14}C-sucrose).

The data reported here support that conclusion because the galactose moiety of the translocated stachyose contained major amounts of ^{14}C. If the translocated ^{14}C-stachyose had been synthesized during or subsequent to phloem loading from exogenous ^{14}C-sucrose and endogenous galactose, little if any ^{14}C should have been incorporated into its galactose moieties. Not only were there significant amounts of ^{14}C in the galactose moieties of the translocated stachyose, but the specific radioactivity of the galactose moiety exceeded that of the glucose moiety.

The conclusion drawn from these data is that the supplied ^{14}C-sucrose followed two pathways. A portion was loaded directly into the phloem and translocated unaltered. The other portion was absorbed by nontranslocating cells which had the capacity to synthesize stachyose. However, this stachyose was not synthesized directly by adding galactose units to the ^{14}C-sucrose. Rather the supplied ^{14}C-sucrose, which entered these nontranslocating cells, presumably mesophyll cells, was degraded into its constituent hexoses, some of which were converted into galactose. These hexoses were then used in stachyose synthesis.

It would seem that this process should have yielded stachyose which was equally labeled in all of its hexose moieties, but such was not the case. A most reasonable explanation for the observed labeling pattern of stachyose is that the ^{14}C-hexose derived from the supplied sucrose entered a general hexose pool. A portion of that hexose was converted to galactose, presumably via the UDP-hexose form. These galactose units were then added to sucrose which was drawn from a relatively large pool. This large sucrose pool would come into isotopic equilibrium much more slowly than would the smaller hexose pools. As a result, the initial specific radioactivity of the sucrose used in stachyose synthesis would be much lower than the galactose used in the synthesis.

The pathway followed by the carbons supplied as ^{14}C-sucrose is represented in Figure 3, which has been modified from Geiger *et al.* (5). The portion of ^{14}C passing through the mesophyll cells represents the site of stachyose synthesis and is necessary to explain the results presented here in conjunction with results previously reported (8); for if the ^{14}C translocated as sucrose and stachyose had followed the same pathway, labeled stachyose should not have lagged behind labeled sucrose, as it, in fact, did (8). In addition, had the stachyose been synthesized in the translocation system, it is difficult to imagine how the translocated sucrose and that sucrose used in stachyose synthesis could have been segregated to yield lower specific radioactivity in the glucose than in the galactose moieties of the stachyose. It follows that the site of stachyose synthesis was not in the main translocation pathway or at the phloem-loading site.

The over-all picture of phloem loading that evolves is that the materials which enter the phloem are loaded from the apoplast. In the intact plant, these materials are supplied by secretion

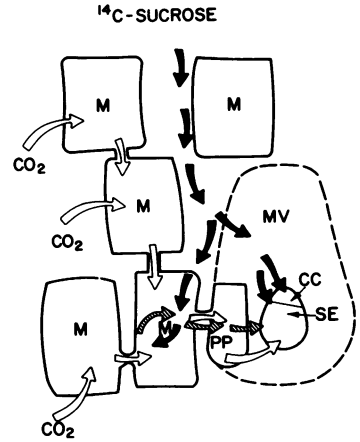

FIG. 3. Proposed loading pathway for ^{14}C from supplied sucrose and for $^{12}CO_2$. Open arrows: ^{12}C; closed arrows: ^{14}C-sucrose; striped arrows: ^{14}C-stachyose; M: mesophyll: PP: phloem parenchyma; MV: minor vein; CC: companion cell; SE: sieve element. Modified from Geiger *et al.* (5). The fact that some items from the original are not included does not imply disagreement, but rather a different emphasis.

from the mesophyll cells. Certainly, the secretion into the apoplast must be selective, for we see that a limited number of compounds are translocated in large quantities. In addition, the phloem-loading process is selective, for not all compounds which are supplied exogenously are translocated with equal facility (11). With some modification, these views are in general agreement with those presented by Geiger *et al.* (5).

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