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Communication

Sucrose Synthase and Invertase in Isolated Vascular Bundles[1]

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ABSTRACT

Vascular bundles were isolated from grapefruit (*Citrus paradisi* Macf.) during periods of rapid sucrose translocation into fruit. Invertase and sucrose synthase activities were assayed in these strands and compared with immediately adjacent tissues (inner most peel and segment epidermis) and phloem-free juice sacs during four growing seasons. Although sucrose synthase was present in sink cells, the significantly greater activity in vascular strands (per unit fresh weight and protein) indicated that the role of this enzyme in translocation may include a vascular function in addition to its proposed involvement in metabolism of importing cells.

The role of sucrose synthase in translocation and sucrose partitioning remains unresolved despite extensive study of its association with elevated carbon import. Although growing interest has centered on its involvement in sucrose metabolism by importing cells (2, 17), additional evidence also supports a possible vascular function (1, 3, 6). Recently, Yang and Russell (19) suggested that the promoter for one of the sucrose synthase genes in maize (shrunken 1 gene) was "phloem specific" in transgenic tobacco plants. However, other studies have indicated expression of sucrose synthase genes may be more complex and appears to be sensitive to changes in metabolism and/or environment (14, 15). Nonetheless, immunohistological evidence has indicated a greater abundance of sucrose synthase protein in vascular areas of young roots (1). The levels of total protein also tend to be greater in vascular tissues (13), however, leaving the extent of sucrose synthase activity in vascular tissues unresolved.

The possibility that sucrose synthase activity may be greater in vascular bundles has been difficult to address because few opportunities exist for isolation of vascular strands (4). Most plant tissues have fine networks of vascular bundles that cannot be effectively separated from adjacent parenchymal cells. Hawker and Hatch (6) initially suggested a vascular localization for sucrose synthase in sugarcane internodes despite nonvascular storage parenchyma making up approximately 70% of their isolated transport tissues. Subsequently, Claussen *et al.* (3) found that sucrose synthase was more active on a fresh weight basis in midribs than in laminae of eggplant leaves but not of sugarcane or maize. Lowell *et al.* (13) reported greater activities of this enzyme in the collective transport tissues of citrus fruit, although vascular strands were not isolated. Sucrose synthase has also been detected in phloem sap from several species (8, 12), but no comparative information was available for adjacent tissues.

Isolated vascular bundles of grapefruit therefore offer an unusual opportunity to compare activities of sucrose metabolizing enzymes in vascular strands with those in adjacent nonvascular structures. In the present study, we tested the hypothesis that sucrose synthase activity was greater in vascular bundles of this system.

MATERIALS AND METHODS

'Marsh' grapefruit (*Citrus paradisi* Macf.) were sampled from the outer, southern canopy of 55-year-old trees in a commercial orchard in Lake Wales, FL, during late August to early September (before completion of the expansive phase of growth) during four consecutive growing seasons. Samples for immediate dissection were also obtained during the fourth growing season from 10-year-old containerized trees grown in Gainesville, FL. Results from these fruit were similar to those from the orchard-grown trees and were included in overall means.

Fruit were washed, sectioned longitudinally, peeled, and separated into individual segments. These were dissected into tissues pictured in Figure 1: (a) dorsal vascular bundle, (b) albedo (inner-most peel), (c) dorsal face of the segment epidermis, and (d) intact juice sacs attached nearest to the dorsal vascular bundle. During the first two seasons, juice sacs were separated into thread-like stalks and multicellular heads. Segment epidermis was divided into 2-mm strips distal and proximal to the dorsal vascular bundle during the last two seasons. Dissections were conducted under 20 mM Mes buffer (pH 6.0) during the first season and on moist filter paper in subsequent years. Tissues were frozen in liquid nitrogen immediately after isolation and stored at −80°C until assay.

Frozen tissue was ground to a fine powder in liquid nitrogen and then transferred to 5 volumes of ice-cold homogenization buffer (200 mM Hepes buffer [pH 7.5] containing 1 mM DTT, 1 mM EGTA, 5 mM $MgCl_2$, 10 mM sodium ascorbate, and 10

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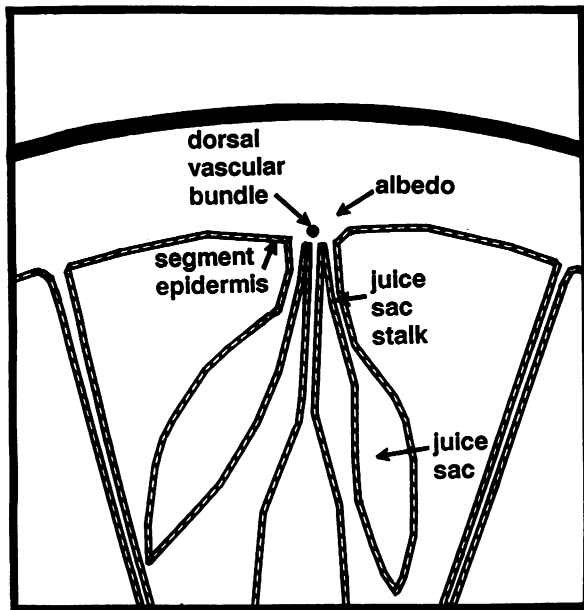

Figure 1. Diagram of positions of vascular and nonvascular tissues along the pathway of assimilate transfer in citrus fruit. Tissues isolated were albedo, dorsal vascular bundle, dorsal face of the segment epidermis, and juice sacs (comprised of stalks and multicellular heads). The fruit exterior (outermost peel) is oriented toward the top of the figure.

mM cysteine-HCl) plus 10% polyvinylpolypyrrolidone. Each sample was further homogenized at low speed with a Polytron (Brinkmann, Westbury, NY) or in a mortar and pestle. Homogenate was filtered through four layers of cheesecloth, and residue was rinsed to obtain a 1:10 (g/mL) ratio of tissue to buffer. Extract was centrifuged (20,000*g*, 4°C) for 20 min and the supernatant dialyzed against one of two buffers. For sucrose synthase, homogenization buffer was diluted 1:40 and used without added sodium ascorbate and cysteine-HCl. For invertase, 25 mM potassium phosphate buffer was used at pH 7.4. Dialysis was performed at 4°C, and buffers were changed four times in 8 h.

Sucrose-metabolizing enzymes were assayed for 15 min at 30°C. Boiled, crude extracts were used to check nonenzymatic activity. Soluble acid invertase was assayed in a 0.6-mL volume containing crude extract, 200 mM sucrose, 100 mM potassium acetate buffer (pH 4.5), and 14.5 mM potassium phosphate buffer (dialysis buffer, pH 7.4). For soluble alkaline invertase, potassium phosphate buffer (pH 7.4) was substituted for the potassium acetate buffer. The reactions were terminated by addition of 0.5 mL of 0.4 M potassium phosphate buffer (pH 7.4) and transfer to a boiling water bath for 3 min. Glucose formation was quantified by the Peroxidase-glucose oxidase method (Sigma). Sucrose synthase (cleavage direction) was assayed in a 0.5-mL volume containing crude extract, 50 mM Mes buffer (pH 5.5), 5 mM EDTA, 5 mM NaF, 5 mM UDP, and 50 mM sucrose as described previously (13). UDPG production was quantified by measurement of uridine diphosphate glucose dehydrogenase-specific synthesis of NADH (13). This method yielded data equivalent to values obtained with a recently developed radiometric assay (5) (data not shown). Data were converted to units of sucrose cleaved.

Protein content of extracts was determined by the bicinchoninic acid method (Pierce Chemical Co., Rockford IL) after precipitating with TCA plus sodium desoxycholate. BSA standard was used.

Statistical analyses were conducted using the MGLH module of SYSTAT (18). Analysis of variance was used to test for differences due to tissue or year for each enzyme; assumptions were checked using correlation analysis, normality plots, and scatter plots. LSD was used to separate means when tissue effects were significant.

RESULTS AND DISCUSSION

A comparison between sucrose-metabolizing enzyme activities in vascular strands and adjacent tissues was made

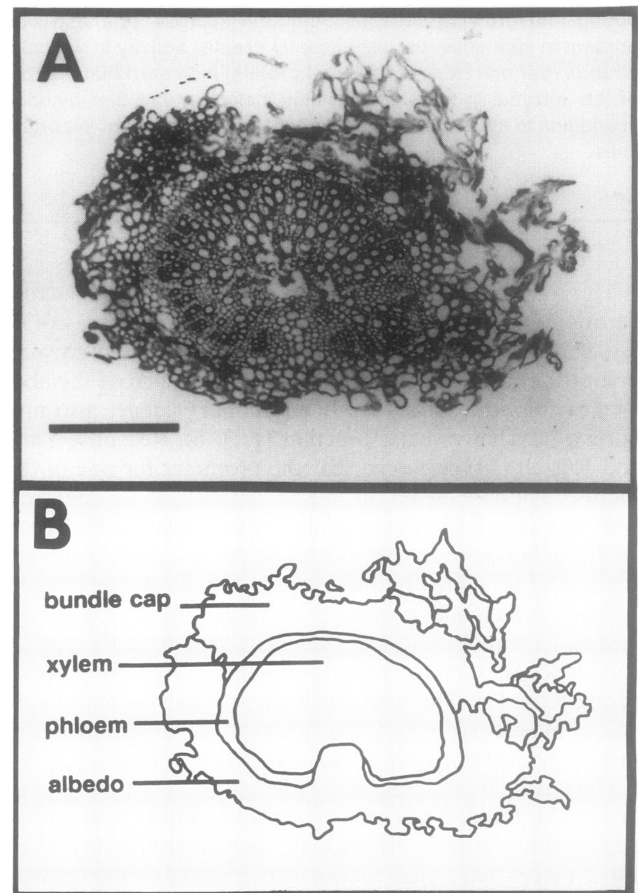

Figure 2. Dorsal vascular bundle from grapefruit. A, Cross-section of an isolated dorsal vascular bundle; bar, 250 μm. B, Diagram showing location of tissues, including the extent of phloem, in these amphicribal strands. Vascular bundles were fixed in FAA (formalin [10%]: glacial acetic acid [5%]: ethanol [48%]), dehydrated through a tertiary butanol series, and embedded in paraffin. Sections, 8 μm, were counterstained with safranin/fast green and permanently mounted for microscopic observation (16). Cross-section is oriented with the side adjacent to the segment epidermis toward the bottom of the figure.

Table I. *Sucrose Synthase and Soluble Invertase Activities in Isolated Vascular Bundles and Sucrose-Importing Tissues (Albedo, Segment Epidermis, and Juice Sacs) of Grapefruit*

Values are means ± SE of the number of replicates given in parentheses, from four growing seasons.

Enzyme	Vascular Bundles	Albedo	Nonvascular Tissues	
			Segment epidermis	Juice sacs
		μmol sucrose degraded·mg protein^{-1}·h^{-1}		
Sucrose synthase[a]	3.5 ± 0.6 (12) a	0 ± 0 (6) b	1.7 ± 0.4 (12) c	2.1 ± 0.4 (12) d
Soluble invertase[b]	0.8 ± 0.3 (10)	0 ± 0 (6)	0.4 ± 0.1 (11)	0.5 ± 0.2 (11)

[a] Enzyme activity data followed by different letters (a–c) are significantly different (P < 0.05). [b] No significant tissue effects were observed for either acid (P = 0.50) or alkaline (P = 0.30) invertase activity. Significant year effects were observed for both invertases (P = 0.003 and 0.005 for acid and alkaline invertases, respectively). Tissue by year interactions were significant for acid (P = 0.005) but not for alkaline invertase (P = 0.43).

possible by isolation of dorsal vascular bundles from adjacent tissues in citrus fruit (positioned as shown in Fig. 1). A small amount of parenchymous albedo tissue generally adhered to the surfaces of these vascular strands (Fig. 2); however, albedo had no detectable sucrose synthase or invertase activity (Table I). Previous work (9–11) indicated that dorsal vascular bundles were a primary avenue of sugar translocation into fruit. Subsequent transfer of sucrose to juice sacs was found to proceed via the completely nonvascular tissues adjacent to bundles (segment epidermis and juice sac stalks) (10).

Sucrose synthase activity per unit protein was greater in the isolated dorsal vascular bundles than in any other fruit tissue assayed (P = 0.02) (Table I). There was no detectable activity of this enzyme in the albedo (inner peel) nearest to the dorsal vascular bundle. Sucrose synthase in segment epidermis and juice sacs was also twofold less active than in dorsal vascular bundles. Total invertase activity was five- to 10-fold less than that of sucrose synthase in all tissues examined and did not differ significantly between vascular and nonvascular areas (Table I). Similarly, sucrose phosphate synthase activity (assayed as described by Lowell *et al.* [13]), did not differ significantly between tissues (P = 0.14) (data not shown).

No significant difference in sucrose synthase activity was observed within nonvascular fruit tissues as a function of distance from the vascular bundle. Although mean activity of sucrose synthase was greater in strips of segment epidermis adjacent to the dorsal vascular bundle, values did not differ significantly (2.5 ± 0.8, mean ± SE [n = 4] and 1.2 ± 0.6 [n = 3] μmol sucrose degraded·mg protein^{-1}·h^{-1}, respectively). Activity in different portions of juice sacs was also similar, with values from the basal parenchymous transport tissues (stalks) varying little from those of the multicellular storage areas (heads) (0.8 ± 0.5 [n = 6] and 1.2 ± 0.4 [n = 6] μmol sucrose degraded·mg protein^{-1}·h^{-1}, respectively).

These data confirm the hypothesis that sucrose synthase activity is locally elevated in vascular tissues of this system and provide evidence at the level of enzyme activity consistent with that of promoter analysis for one of the sucrose synthase genes (19) and of immunohistology (1) in other species. Previous attempts to obtain such information (3, 6, 8, 12) have been complicated by potential differences in tissue protein levels, incomplete isolation of vascular strands, and/or a lack of comparative data from adjacent tissues.

The physiological significance of elevated sucrose synthase activity in vascular bundles may be related either to phloem unloading or to processes involved in general phloem functioning. Sucrose cleavage by sucrose synthase in cells immediately outside the translocation path may help establish or maintain a descending sucrose gradient considered favorable for sucrose exit from the phloem (7). Alternatively, sucrose synthase, catalyzing a reversible reaction, may play some role in maintaining an equilibrium between sucrose and its breakdown products in the cells adjacent to sieve tube elements (3). In contrast, sucrose synthase activity in or near the phloem may provide substrate for the greater metabolic rates needed to support active phloem unloading or reloading, energy-dependent aspects of symplastic transfer, and/or greater respiratory demands than in storage parenchyma cells (7).

The suggestion that sucrose synthase activity is a good indicator of sink strength (2, 17) thus should be modified or refined to account for the locally elevated activity of sucrose synthase in vascular bundles of this (Table I) and probably other systems (1, 3, 8, 12, 19). Although sucrose synthase is present in sink cells of citrus fruit (Table I), its significantly greater activity in vascular strands indicates a vascular function in addition to its proposed involvement in metabolism of importing cells. The possibility remains that respiratory activity of vascular cells could result in a substantial requirement for sucrose and a cellular sink demand within the transport path.

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