Carbohydrate Content and Enzyme Metabolism in Developing Canola Siliques

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Little biochemical information is available on carbohydrate metabolism in developing canola (Brassica napus L.) siliques (pod) wall and seed tissues. This research examines the carbohydrate contents and sucrose (Suc) metabolic enzyme activities in different aged siliques wall and seed tissues during oil filling. The siliques wall partitioned photosynthate into Suc over starch and predominantly accumulated hexose. The siliques wall hexose content and soluble acid invertase activity rapidly fell as embryos progressed from the early- to late-cotyledon developmental stages. A similar trend was not evident for alkaline invertase, Suc synthase (SuSy), and Suc-phosphate synthase. Siliques wall SuSy activities were much higher than source silique wall activities. With the switch to oil accumulation, seed Suc activity increased by 3.6-fold and soluble acid invertase activity decreased by 76%. These data provide valuable baseline knowledge for the genetic manipulation of canola seed carbon partitioning.

The sources of assimilate for developing seeds of canola (Brassica napus L.; Brassica rapa L.) have not been clearly elucidated. During the life of a plant there is a clear sequence of developmental phases that proceed from leaf to stem to silique (pod) to seed (Mendham and Salisbury, 1995). Leaf photosynthesis provides assimilate for the growth of shoot and root meristems. At the initiation of reproductive growth, there is a rapid increase in flower-bearing branches from the shoot apical meristem. The photosynthetic leaf area then quickly declines because of senescence (Pechan and Morgan, 1985), thereby removing one source of assimilate at a time when seeds have a great import demand. At this time, only the oldest seeds at the base of a plant would have begun storage product synthesis. In the absence of leaves, siliques wall photosynthesis is the main source of assimilates during this growth phase and may contribute up to 50 to 60% of final plant dry matter (Lewis and Thurling, 1994).

Like other dicotyledonous plants, canola produces seed storage products in the embryo (Murphy and Cummins, 1989). Early in development, the embryo is very small and the main seed constituents are the testa and liquid endosperm (Downey and Fowler, 1970). During these initial stages embryo cells are rapidly dividing. At the early- to midcotyledon stages (Pomeroy et al., 1991), embryo cells begin to rapidly expand, the resulting growth consumes the liquid endosperm, and the embryo fills the seed’s internal space (Downey and Fowler, 1970). Coincident with rapid embryo growth, storage oil accumulates and peaks at maximum fresh weight (Rakow and McGregor, 1975; Murphy and Cummins, 1989; Hocking and Mason, 1993). There is a delay after oil accumulation initiation before the storage proteins accumulate (Crouch and Sussex, 1981; Murphy and Cummins, 1989).

Starch and Suc are the major end products of photosynthetic carbon fixation. Starch is synthesized and temporarily stored in the chloroplast for later remobilization and use. To synthesize Suc, triose-phosphate is exported to the cytosol, where it is converted through a series of enzyme-mediated reactions. The rate of Suc formation is primarily regulated by Fru-1,6-bisphosphatase (EC 3.1.3.11) and SPS (EC 2.4.1.14) (Stitt et al., 1987). In most species Suc is the preferred form of carbon that is exported via the phloem to sink tissue. Suc may be cleaved in the apoplast by cell wall-bound acid invertase, in the cytosol by SuSy or alkaline invertase, or in the vacuole by soluble acid invertase. The products of Suc cleavage are converted to hexose phosphates and can enter the respiratory pathways via glycolysis to provide substrates and reducing power for growth and storage product synthesis. Acetyl-CoA is the immediate precursor for fatty acid synthesis, and amino acids are needed for storage protein formation.

This study has focused on the growth and development of source siliques wall and its developing seed sinks during the oil-filling period. Photosynthetic carbon partitioning, carbohydrate content, and Suc metabolic enzymes have been measured and compared at the beginning, middle, and end of this period. The objective was to identify key elements of source-sink carbohydrate metabolism in canola siliques.

Abbreviations: DAA, days after anthesis; DAFF, days after first flower; SPS, Suc-P synthase; SuSy, Suc synthase.
MATERIALS AND METHODS

Plants of canola (Brassica napus L. cvs Westar and Hyola 42) were grown in a mixture of compost and perlite (1:1, v/v) supplemented with Osmocote slow-release fertilizer (Scotts, Nedlands, Australia). Plants were grown in a naturally illuminated greenhouse with temperatures set at 23°C/18°C day/night. At floral initiation emerging flowers were tagged three times weekly in the early morning. Only siliques from the main raceme and the first two branches were used for the experiments. All plants were well spaced to maximize light interception and to minimize canopy effects.

All biochemicals and enzymes were supplied by Boehringer Mannheim or Sigma. Barium [14C] carbonate was obtained from Amersham. All other reagents were of an analytical grade.

Leaf Area Measurements

At weekly intervals following the opening of the first flower, leaf area was individually measured for all leaves from each of the four plants. Each leaf outline was traced onto paper, cut out, and weighed on an electronic balance. Paper weights were converted to leaf area using a standard curve. Each leaf was visually scored for its color, and only leaves that had lost all green pigmentation were counted as being senescent.

14CO2 Partitioning

Leaves and siliques were pulse-labeled with 14CO2, as described by Lunn and Hatch (1995). Two leaf pieces of approximately 25 cm² were cut around the mid-rib of young, fully-expanded leaves before placing the basal end in a water-filled trough of a Perspex chamber (Lunn and Hatch, 1995). The cut petiole ends of four siliques were placed in the water-filled trough. Tissues were illuminated for 30 min at an irradiance of 1000 to 1200 μmol quanta m⁻² s⁻¹, 400 to 420 μL L⁻¹ CO₂, and 25°C to reach steady-state photosynthetic rates before the injection of 14CO2 into the sealed chamber. After a 135-s pulse, leaves were removed and killed in boiling 80% (v/v) ethanol for 1 min. Siliques were removed after 600 s and plunged into liquid nitrogen. Gentle crushing with a pestle allowed separation of the silique wall and seed tissues before boiling in 80% (v/v) ethanol. The rest of the extraction procedure and analysis was identical to Lunn and Hatch (1995).

Carbohydrate and Lignin Analysis

Tissue samples were taken from three plants (replicates) just before sunrise (6 AM) and after 12 h (6 PM) during a partly sunny day in late spring. The day’s accumulated PAR was 29.9 mol quanta m⁻² as measured inside of a greenhouse. Immediately after harvest, samples were frozen in liquid nitrogen and stored at −80°C until analysis. Each leaf sample contained three 1.33-cm² leaf discs from the youngest fully expanded leaf on plants sown 1 month previously. Each silique sample contained two intact siliques of the same age.

Siliques were removed after 600 s and plunged into liquid nitrogen and stored at −80°C until analysis. Siliques were gently crushed in liquid nitrogen to separate silique wall and seed tissue. Samples of the silique wall, containing approximately 40 μg of chlorophyll, or 15 seeds were extracted in 1.5 mL of the extraction buffer, which contained 50 mM Hepes-KOH, pH 7.5, 10 mM MgCl₂, 1 mM EDTA, 1 mM EGTA, 1 mM benzamidine, 5 mM e-aminocaproic acid, 0.5% (v/v) BSA, 2% (w/v) insoluble PVP, 0.1% (v/v) Triton X-100, 2 mM DTT, 2 μM leupeptin, 2 μM antipain, 1 mM PMSF, and 1 mM benzamide. A 100-μL sample of the extract was added to 1 mL of cold
methanol for chlorophyll determination (Porra et al., 1989). The remaining extract was centrifuged for 2 min at 12,000g, and a 0.5-mL sample of the supernatant was desalted by passage through a 4-mL Sephadex G-25 column (Pharmacia) pre-equilibrated with the extraction buffer minus BSA, insoluble PVP, and Triton X-100. All procedures were done at 4°C. Enzymes were assayed immediately in duplicate.

**SPS (EC 2.4.1.14)**

Total SPS activities were assayed by measuring the synthesis of Suc-6-P (and Suc) from UDP-Glc and Fru-6-P (Huber and Huber, 1991). Each reaction contained 20 mM UDP-Glc, 5 mM Fru-6-P, 17.5 mM Glc-6-P, and 50 µL of extract in a total volume of 100 µL. The reaction was started by the addition of extract and incubated at 25°C for 10 min. After stopping the reaction with 100 µL of 5 M KOH and 10 min of heating at 100°C to destroy unreacted hexoses and hexose phosphates, 1 mL of 0.14% (w/v) anthrone in H₂SO₄ was added before 40 min of incubation at 40°C. Suc-6-P (and Suc) content was determined by relating the A₆28 to that of a standard curve (0-200 nmol of Suc). The recovery of Suc was estimated by incubating 50 µL of extract with 100 nmol of Suc under the above assay conditions.

**SuSy (EC 2.4.1.13)**

UDP-dependent cleavage of Suc into UDP-Glc and Fru was assayed (Copeland, 1990). Each reaction contained 20 mM Pipes-KOH, pH 6.5, 100 mM Suc, 2 mM UDP, and 20 µL of extract in a total volume of 250 µL. Control reactions lacked UDP. Reactions were started by the addition of extract and incubated at 25°C for 30 min. The reactions were stopped with 250 µL of 0.5 M Tricine-KOH, pH 8.3, and boiling for 10 min. Fru was measured spectrophotometrically, as described above.

**Invertases (EC 3.2.1.26)**

Soluble acid and alkaline invertases were measured by incubation of 20 µL of extract with 100 mM Suc in 100 mM acetic acid-NaOH, pH 5.0 (acid invertase), or 100 mM sodium acetate-acetic acid, pH 7.5 (alkaline invertase), in a total volume of 250 µL. Reactions were started by the addition of extract and incubated at 25°C for 30 min. The reactions were stopped with 250 µL of 0.5 M Tricine-KOH, pH 8.3, and boiling for 10 min. Control reactions contained boiled extract, and Glc was measured spectrophotometrically, as described above.

**RESULTS**

As a first step in determining the important elements of silique carbon metabolism, the potential contribution from leaves was assessed by measuring leaf area after the emergence of the first flower. At weekly intervals, leaf area was measured nondestructively and leaf color was used as an indicator of photosynthetic capacity. Fully yellow leaves were classified as being senescent. By first flower, 40% of total leaf area was already senescent and all leaves were senescent by 35 DAFF (Fig. 1). By 35 DAFF, the seed age on a plant ranged from approximately 14 to 35 DAA due to canola's indeterminate growth habit. Maximum storage oil accumulation occurred 23 to 32 DAA (S.P. King and R.T. Furbank, unpublished data) as embryos progressed from early- to late-cotyledon development stages (Pomeroy et al., 1991).

Incorporation of 14CO₂ into the primary photosynthetic end products, Suc and starch, was measured after a short pulse under steady-state physiological conditions. Radioactivity in Suc and aqueous-ethanol-insoluble fractions was used to calculate the partitioning of photosynthate between Suc and starch. Within the 1st h of illumination both source leaves and siliques preferentially partitioned newly fixed carbon into Suc (Table I). Near the end of the photoperiod, leaves were producing more starch than Suc, whereas siliques continued to partition more photosynthate into Suc. At both times, silique Suc-to-starch partitioning ratios were 3- to 4-fold higher than leaves. Negligible radioactivity was found in seeds, and in all other tissues hexoses contained less than 2.5% of total radioactivity.

Carbohydrate accumulation in silique wall and seed tissues was examined during the progression from embryo early- to late-cotyledon stages. All samples were taken on a single day from plants sown on the same day (plants for leaf samples sown later). To guard against a position effect, only the main raceme and the first two branches were used for sampling; samples for all of the experiments reported in this paper were taken from plants aged 30 to 40 DAFF. The contents of hexose, Suc, and starch of the developing siliques are presented in Figure 2. In the silique wall the predominant carbohydrates were Glc and Fru (Fig. 2A). With age, hexose levels fell rapidly. Although present in a smaller quantity, starch also decreased with development, whereas Suc levels were essentially stable. In seeds the predominant carbohydrate was starch (Fig. 2B). Along with Suc, starch levels did not significantly change in the 21 to 35 DAA period. Hexoses did, however, drop significantly between 21 and 28 DAA. This period corresponds to the beginning of rapid embryo fresh weight gains (S.P. King

![Figure 1](https://www.plantphysiol.org/)

**Figure 1.** Increase of senescent leaf area after first flower. Fully yellow leaves were scored as senescent and their areas are expressed as a percentage of total leaf area at first flower. Individual leaves did not increase in area after this time.
endosperm (Table 111). It was difficult to separate very 

between the testa/endosperm and the embryo, whereas the 

therefore, some of the hexose and starch in the embryo 

endosperm before a separate analysis for hexoses, SUC, and 

starch. On a dry weight basis, Suc was evenly distributed 

samples may have come from liquid endosperm adhesion. 

samples were taken near the end of the photoperiod (6 

There were no significant differences between the morning 

active leaves (Table 1). 

Silique wall secondary cell wall thickening may be an 

of three replicates are plotted for each measured carbohy-

Photosynthate partitioning in canola leaves and siliques 
The relative rates of $^{14}$CO$_2$ incorporation into Suc and starch were measured in leaves and siliques undergoing steady-state photosynthesis at an irradiance of 1000 to 1200 μmol quanta m$^{-2}$ s$^{-1}$, 400 to 420 μmol L$^{-1}$ CO$_2$, and 25°C. Measurements were made in duplicate 1 and 9 h after the start of the photoperiod.

Table I. Photosynthate partitioning in canola leaves and siliques

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Age</th>
<th>Photosynthate Partitioning ($^{14}$C) Suc to $^{14}$C Starch</th>
<th>1 h</th>
<th>9 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Fully expanded</td>
<td></td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Silique</td>
<td>21 DAA</td>
<td>Recovered Suc (μmol hexose equivalents g$^{-1}$ chlorophyll)</td>
<td>5.7</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>35 DAA</td>
<td>Recovered Starch (μmol hexose equivalents g$^{-1}$ chlorophyll)</td>
<td>6.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

and R.T. Furbank, unpublished data). The data presented 

in Figure 2 are drawn from samples that were taken at the 

beginning of the photoperiod (6 AM). An equal number of 

samples were taken near the end of the photoperiod (6 PM). 

There were no significant differences between the morning 

and evening samples in either the silique wall or seed. As 

a comparison, starch increased 5-fold in photosynthetically 

active leaves (Table II).

To investigate carbohydrate distribution within young 

seeds, embryos were dissected from the testa and liquid 

endosperm before a separate analysis for hexoses, Suc, and starch. On a dry weight basis, Suc was evenly distributed 

between the testa/endosperm and the embryo, whereas the 

hexoses and starch were much more prevalent in the testa/ 

endosperm (Table III). It was difficult to separate very 

small embryos completely from the liquid endosperm; 

therefore, some of the hexose and starch in the embryo 

samples may have come from liquid endosperm adhesion. 

Asymmetric carbohydrate distribution may be more 

pronounced than the data in Table III indicate.

Silique wall secondary cell wall thickening may be an 

additional sink for carbon during development. Secondary 

cell walls are comprised of cellulose, lignin, hemicellulose, 

and pectin (Aspinall, 1980). To estimate the importance of 

secondary cell wall thickening, cellulose and lignin con-

Table II. Diurnal carbohydrate accumulation in canola source 

leaves

Samples were taken at 6 AM (sunrise) and 6 PM from plants grown 
in a naturally illuminated greenhouse. Accumulated PAR for the day 
was 29.9 mol quanta m$^{-2}$. Values are expressed as mean values ($\pm$SE) 
from three replicates.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Carbohydrate Content</th>
<th>Diurnal Accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 AM</td>
<td>6 PM</td>
</tr>
<tr>
<td>Starch</td>
<td>12.5 (1.0)</td>
<td>73.2 (19.7)</td>
</tr>
<tr>
<td>Glc</td>
<td>13.0 (2.4)</td>
<td>19.8 (5.3)</td>
</tr>
<tr>
<td>Fru</td>
<td>12.0 (2.3)</td>
<td>17.7 (5.2)</td>
</tr>
<tr>
<td>Suc</td>
<td>4.1 (1.3)</td>
<td>4.6 (1.0)</td>
</tr>
</tbody>
</table>

To investigate the pathways of carbohydrate metabo-

lism, the activities of key enzymes of Suc metabolism were measured in silique wall, seed, and leaf tissues. Care was taken to prevent proteolysis during extraction by including a variety of protease inhibitors in the extraction buffer. All extraction procedures were done quickly at 4°C followed immediately by the enzyme assays. Assay conditions were pre-optimized for each tissue to ensure that saturating 
substrate concentrations were used, and that the rate of product formation was linear with respect to time and the amount of extract assayed. For SPS Suc recoveries after incubation with extracts were greater than 92%, showing that measurement of SPS activity was not significantly affected by Suc losses during the reaction. In the SuSy 
assays control reactions without UDP were used to correct 
for any hexose production via invertases. The absence of UDP in desalted extracts would prevent a SuSy-dependent cleavage of Suc in the invertase assays.

Table III. Carbohydrate localization in canola seeds containing 

early-cotyledon embryos

Parallel triplicate samples were used to measure carbohydrates 

and dry weight. SSEs ranged from 2 to 23%.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Carbohydrate Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testa/Endosperm</td>
</tr>
<tr>
<td></td>
<td>μmol hexose equivalent g$^{-1}$ dry wt</td>
</tr>
<tr>
<td>Starch</td>
<td>939</td>
</tr>
<tr>
<td>Glc</td>
<td>128</td>
</tr>
<tr>
<td>Fru</td>
<td>145</td>
</tr>
<tr>
<td>Suc</td>
<td>312</td>
</tr>
</tbody>
</table>

Figure 2. Carbohydrate content of developing silique wall (A) and 
seed (B). Samples were taken at sunrise on a single day. Mean values 
and siks of three replicates are plotted for each measured carbohy-

date and age, chl, Chlorophyll.
activity was 1.2- to 1.5-fold higher than SPS, whereas in leaves SuSy activity was barely detectable. Soluble acid invertase activity in the silique wall was initially higher than alkaline invertase activity, but dropped markedly between 22 and 26 DAA. The timing of this drop corresponded to the reduction in silique wall hexose content (Fig. 2A). Enzyme activities in developing seeds (Fig. 3C) are expressed on a seed basis because chlorophyll content increased dramatically during the sampling period (0.8 µg seed⁻¹ at 22 DAA, 1.4 µg seed⁻¹ at 26 DAA, and 1.5 µg seed⁻¹ at 30 DAA). In young seeds, in which embryos were at the early-cotyledon stage (22 DAA), the activities of the four measured enzymes were similar. As embryo cells rapidly gained fresh weight and developed to the mid- and late-cotyledon stages, total SuSy activity increased 3.6-fold (26–30 DAA). At the same time, soluble acid invertase activity declined linearly. SPS and alkaline invertase activities increased between 22 and 26 DAA and then declined again by 30 DAA.

**DISCUSSION**

**Silique Wall Metabolism**

The rapid decline of functional leaf area after floral initiation (Fig. 1) removes a major source of photoassimilate to developing seeds. All leaves were senescent by 35 DAFF, which is before the completion of storage product synthesis in the bulk of a plant’s seeds. The silique wall and possibly stem tissues must therefore take over carbon provision. The silique wall has a photosynthetic capacity similar to or greater than that of the leaf on a chlorophyll basis, but a 75 to 80% lower chlorophyll concentration reduces photosynthesis per unit area (S.P. King and R.T. Furbank, unpublished data). Unlike many starch-storing leaves of C₃ dicotyledonous species, the canola silique wall preferentially partitions photosynthetic Suc into SUC (Table I). A portion of this Suc appears to be hydrolyzed by acid invertase to hexose for vacuolar storage before rapid seed growth (Figs. 2A and 3B). Unlike the source leaf (Table II), there was no detectable diurnal increase in silique wall carbohydrates, thereby suggesting balanced synthesis and utilization during the day and little utilization at night.

Silique wall hexose storage is only temporary, because contents dropped markedly at the onset of rapid seed growth (Fig. 2A). Although present in lesser amounts, starch also declined over this period. These trends suggest the remobilization of silique wall carbon to seeds. The parallel timing of silique wall carbon loss and rapid seed growth has been previously observed under different growth conditions (Norton and Harris, 1975; for review, see Mendham and Salisbury, 1995). In addition, silique wall carbon reserves can be used for internal metabolic events, as suggested by the continued high SuSy activities 22 to 30 DAA (Fig. 3B). Silique wall cellulose and lignin contents increased 21 to 35 DAA, indicating secondary cell wall thickening (Table IV). In addition, other carbon compounds such as hemicellulose and pectin are involved in thickening (Aspinall, 1980). There are, therefore, simultaneous large carbon requirements for secondary cell wall synthesis and rapid seed growth. We propose that this carbon is drawn from a silique wall Suc pool derived from photosynthesis, import (i.e. younger siliques and stem), and hexose reserves. This Suc pool can be depleted by a combination of export to seeds and SuSy or alkaline invertase-cleavage to fuel secondary cell wall thickening and protein synthesis. Although silique wall Suc contents were always low (Fig. 2A), continuous input and output is not reflected by this type of measurement. In the absence of leaves (Fig. 1), silique wall tissue must be a major supplier of carbon to developing seeds.

**Seed Metabolism**

In this paper seed carbohydrate metabolism has been examined during early-, mid-, and late-cotyledon stages corresponding to maximum fatty acid accumulation (Pomeroy et al., 1991). Imported Suc is likely to be the predominant carbon source for seed growth. At 21 DAA,
seed starch content was higher than Suc or hexose (Fig. 2B). Embryos were at the midcotyledon stage by 28 DAA and had filled the seed by 35 DAA (S.P. King and R.T. Furbank, unpublished data). Starch and hexose in seeds containing early-cotyledon embryos were localized to the testa or liquid endosperm (Table III). In contrast, Suc was evenly distributed between seed fractions. Seed starch is a temporary carbon reserve during early development and is depleted early in the filling phase (Norton and Harris, 1975; Munshi and Kochhar, 1994). Specific localization of starch in canola seeds is unresolved. In pea there is a transitory starch accumulation in testa parenchyma cells until rapid embryo growth (Rochat and Boutilin, 1992), however, the content was not compared with endosperm.

Although starch was the predominant carbohydrate in seeds (Fig. 2B), the quantity is insufficient to fulfill oil synthesis requirements. Norton and Harris (1975) reported that total oil was 5-fold higher than the peak starch content. Clearly, reserves must be supplemented by continued Suc import and possibly seed CO₂ fixation. In addition to measured soluble sugars and starch, seeds may contain other potential reserve carbohydrate sources, such as pectins, hemicellulose, and sucrosyl-oligosaccharides. It is doubtful that nonfructan sucrosyl-oligosaccharides are present in appreciable quantities from 21 to 35 DAA because equimolar quantities of Glc and Fru were released after invertase treatment of aqueous-phase samples. Invertase cleavage of common nonfructan sucrosyl-oligosaccharides would release either a single Glc or Fru unit, but would leave the remaining oligosaccharide intact (for structures, see Keller, 1989).

Hexose contents of seeds containing early-cotyledon embryos were higher than that of seeds containing mid- to late-cotyledon embryos (Fig. 2B). This early presence of hexose has been noted previously in canola (Norton and Harris, 1975; Titttonel et al., 1995) and faba bean (Vicia faba L.) (Heim et al., 1993; Weber et al., 1995). In faba bean activities of a testa cell wall-bound acid invertase were also high early in development (Weber et al., 1995). Expression of the corresponding gene was localized to the thin-layer parenchyma cells associated with apoplastic unloading. An apoplastic step is required because there is no symplastic connection between the maternal seed coat and the filial embryo. It was therefore postulated that imported Suc unloaded in the testa is hydrolyzed by acid invertase while being transferred to the endosperm (for review, see Patrick and Offler, 1995). Suc cleavage would maintain import by establishing a favorable Suc concentration gradient and by increasing the endosperm water potential. The young embryo could then take up hexose from the endosperm. Protoplasts made from sink cells of several species have been shown to favor hexose uptake over Suc (for review, see Ho, 1988). Hexose was localized to faba bean endosperm and embryo, with minimal amounts in the testa (Weber et al., 1995).

As canola embryos began the transition from cell division to cell expansion (21–28 DAA), seed hexose dropped (Fig. 2B). In faba bean young embryos cultured without hexose stopped cell division and initiated expansion (Weber et al., 1996). Similarly, pea embryos cultured on high Suc favored cell expansion (Ambrose et al., 1987). Hexose seems to inhibit SuSy activity (Heim et al., 1993; Weber et al., 1996). On the disappearance of hexose from developing canola seeds (Fig. 2B), SuSy activities rapidly increased, whereas the other enzymes measured did not change as dramatically (Fig. 3C). Compared with a number of species, SuSy was found to be high in active sinks, but not in quiescent sinks (Sung et al., 1989). No similar correlation with sink type was found with six glycolytic enzymes and soluble invertase activities were low in both sink types.

SuSy activity has been positively correlated with storage product synthesis (Chourey and Nelson, 1976; Edwards and ap Rees, 1986; Doehlert, 1990; Heim et al., 1993; Weber et al., 1995; Zrenner et al., 1995; Ross et al., 1996). In each of these cases, a starch-storing sink was examined. It is interesting that in canola SuSy also mirrored storage product synthesis, even though seeds are a predominantly oil-storing sink. SuSy activity was much lower in the early stages, when starch was accumulating, compared with activities after the switch to oil deposition. Using statistical cluster analysis, SuSy clustered with starch and ADP-Glc pyrophosphorylase in starch-storing maize endosperm (Doehlert, 1990). Instead of SuSy, oil clustered with hexokinase activity in oil-storing maize embryos (Doehlert, 1990). The implication is that invertase supplied carbon for oil synthesis because hexokinases are required to convert free hexose to hexose phosphate. Mature maize kernel contains 66% starch and 4% oil (Doehlert, 1990), whereas mature canola seed contains 54% oil and an insignificant amount of starch (Murphy and Cummins, 1989). It appears that SuSy activity reflects the synthesis of the predominant storage product regardless of its form; starch in grains and oil in oilseeds. Compared with invertase, SuSy-mediated cleavage conserves ATP, and its bidirectional capability may allow for finer metabolic control.

Estimates were made to assess the Suc flux needed to support oil synthesis in canola seeds. From Murphy and Cummins (1989) it was calculated that embryos form 0.13 mg oil d⁻¹ during rapid accumulation. Based on this deposition rate and the mature seed composition of fatty acid types (Murphy and Cummins, 1989), 410 nmol of total fatty acid and 140 nmol of glycerol 3-P would be needed daily. Assuming that the required 3.7 μmol of acetyl-CoA, 3.3 μmol of ATP, and 6.6 μmol of NADPH is entirely supplied by Suc flux through the pentose phosphate pathway, glycolysis, and the pyruvate dehydrogenase complex, 1.6 μmol of Suc would be needed daily. An additional 0.03 μmol of Suc would satisfy the glycerol 3-P requirements. Although oil synthesis is the predominant carbon sink, some carbon will be used for cell wall and protein synthesis, and, therefore, calculated Suc requirements are a minimum. From maximum extractable activities at 26 DAA (Fig. 3C), SuSy and alkaline invertase are in excess and, therefore, could metabolize the necessary carbon, but soluble acid invertase activity is insufficient. The much higher SuSy activity and its developmental timing suggests that it plays a major role in providing carbon for oil synthesis.
Developing seeds had appreciable total SPS activities at all measured stages (Fig. 3C). Suc synthesis via SPS could have two possible roles in canola seed sinks. First, SPS could catalyze Suc formation from newly fixed CO₂ as in source tissues (Stitt et al., 1987). Although developing embryos are capable of in vitro photosynthesis (Eastmond et al., 1996), net embryo CO₂ fixation under the low light conditions within the silique in vivo would presumably be inconsequential compared with Suc import. Second, SPS could resynthesize Suc from hexose produced by cleavage of imported Suc. A continuous cycle of synthesis and degredation in sink tissues has been previously described (Dancer et al., 1990; Wendler et al., 1990; Geigenberger and Stitt, 1991) and could regulate metabolite supply for sink growth. In such a cycle small changes in enzyme activity can markedly alter the rate and direction of net flux (Wendler et al., 1990). In canola seeds synthesizing oil, the ratio of total cleavage and synthetic enzyme activities suggest that the bulk of imported Suc is cleaved by SuSy and alkaline invertase to be used in respiration, whereas a small proportion of the resulting hexose will be recycled by SPS. In contrast to invertase, SuSy-mediated Suc cleavage produces UDP-Glc and Fru and would require less energy to resynthesize Suc via SPS in a futile cycle.

In summary, developing canola seeds appear to store imported Suc transiently as starch and hexose outside of the embryo before storage product synthesis. Embryos likely take up endosperm hexose initially to support cell division and then the transition to cell expansion and storage product synthesis. Once embryos have consumed the liquid endosperm and filled the seed’s internal space, imported Suc could possibly be transferred intact to the embryo via transfer cells (Patrick and Offier, 1995) before cleavage by SuSy. The bulk of hexose would be converted to hexose-phosphate to feed into glycolysis. Flux to respiration may be modulated by a continuous cycle of Suc cleavage and resynthesis.

ACKNOWLEDGMENTS

Seeds used in this study were provided by D.J. Murphy (John Innes Centre, Norwich, UK) and G. Buza (Pacific Seeds, Toowoomba, Australia).

Received October 29, 1996; accepted January 21, 1997.
Copyright Clearance Center: 0032–0889/97/114/0153/08.

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