Changes in carbohydrate metabolism and assimilate export in starch-excess mutants of *Arabidopsis*

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**ABSTRACT**

The aim of this work was to investigate the effects on carbohydrate metabolism of a reduction in the capacity to degrade leaf starch in *Arabidopsis*. The major roles of leaf starch are to provide carbon for sucrose synthesis, respiration and, in developing leaves, for biosynthesis and growth. Wild-type plants were compared with plants of a starch-excess mutant line (*sex4*) deficient in a chloroplastic isoform of endoamylase. This mutant has a reduced capacity for starch degradation, leading to an imbalance between starch synthesis and degradation and the gradual accretion of starch as the leaves age. During the night the conversion of starch into sucrose in the mutant is impaired; the leaves of the mutant contained less sucrose than those of the wild type and there was less movement of ^14^-C-label from starch to sucrose in radio-labelling experiments. Furthermore, the rate of assimilate export to the roots during the night was reduced in the mutant compared with the wild type. During the day however, photosynthetic partitioning was altered in the mutant, with less photosynthetic partitioned into starch and more into sugars. Although the sucrose content of the leaves of the mutant was similar to the wild type during the day, the rate of export of sucrose to the roots was increased more than two-fold. The changes in carbohydrate metabolism in the mutant leaves during the day compensate partly for its reduced capacity to synthesize sucrose from starch during the night.

**Key-words:** *Arabidopsis thaliana*; assimilate partitioning; chloroplasts; photosynthesis; starch degradation; starch synthesis; sucrose.

**INTRODUCTION**

Many plants accumulate transitory starch in their leaves. It is synthesized directly from photosynthetically fixed carbon dioxide and serves as a short to medium term carbohydrate reserve. Transitory starch is degraded during the night, or when the rate of photosynthesis is low, to provide substrates for respiration and for the synthesis of sucrose and other translocated metabolites (Stitt 1984; Fondy, Geiger & Servaites 1989; Servaites, Fondy & Geiger 1989).

The extent to which starch accumulates in leaves differs between species. In *Arabidopsis thaliana* it is the major carbohydrate accumulated and is synthesized throughout the photoperiod (Caspar, Huber & Somerville 1985; Zeeman *et al.* 1998). The importance of storing carbohydrate as starch is reflected in the growth of plants that are unable to synthesize or to degrade fully their transitory starch. The starchless *Arabidopsis* lines *pgm and adg1* (lacking plastidial phosphoglucomutase activity and ADPglucose pyrophosphorylase activity, respectively) grow more slowly in day/night conditions than the wild type, although under continuous illumination the growth rate is equivalent to that of the wild type (Caspar *et al.* 1985; Lin *et al.* 1988a). The growth rate of sex mutants (for starch excess), with a reduced capacity to mobilize starch, is similarly affected (Caspar *et al.* 1991; Zeeman *et al.* 1998).

Disrupting leaf-starch metabolism affects the way that *Arabidopsis* plants develop as well as the rate of growth. Starchless and reduced-starch lines grown under day/night conditions flower at a later developmental stage than the wild type (Bernier *et al.* 1993; Corbesier, Lejeune & Bernier 1998). This is also true for the starch excess line *sex1* (Eimert *et al.* 1995). Furthermore, the shoot-to-root ratio of starchless mutants is decreased with respect to the wild type (Schulze *et al.* 1991). It seems likely that the disruption of starch metabolism leads to changes in the pools of key metabolites and that such changes are perceived by signal transduction pathways involved in controlling the overall growth and development of the plant (Schulze *et al.* 1994).

To provide more information about the way in which starch storage and mobilization influence growth and development we have studied the carbohydrate metabolism of two mutants that have reduced rates of starch mobilization at night. The *sex1* mutant lacks the ability to export glucose, produced by the hydrolysis of starch, from the chloroplast (Trethewey & ap Rees 1994). The *sex4* mutant is deficient in a chloroplastic endoamylase, reducing the rate of starch mobilization (Zeeman *et al.* 1998), and is the focus for the work described here. We show that both mutants (*sex4* and *sex1*) accumulate starch by gradual accretion as the leaves age and present evidence that, in *sex4*, there are significant changes in the photosynthetic
metabolism and assimilate export that compensate in part for the deficiency in starch degradation at night.

MATERIALS AND METHODS

Materials

All enzymes were obtained from Boehringer-Mannheim UK (Lewes, East Sussex, UK), except invertase which was supplied by Sigma Chemical Co. (Poole, Dorset, UK). Radiochemicals were purchased from Amersham International (Amersham, Bucks, UK). Plants were grown in soil as described by Zeeman et al. (1998). Hydroponically grown plants were grown in a mineral nutrient solution containing 5 mol m$^{-3}$ KNO$_3$, 2.5 mol m$^{-3}$ KH$_2$PO$_4$, 2 mol m$^{-3}$ MgSO$_4$, 2 mol m$^{-3}$ Ca(NO$_3$)$_2$, 50 mol m$^{-3}$ Fe.EDTA, 70 mol m$^{-3}$ H$_2$BO$_3$, 14 mol m$^{-3}$ MnCl$_2$, 0.5 mol m$^{-3}$ CuSO$_4$, 1 mol m$^{-3}$ ZnSO$_4$, 0.2 mol m$^{-3}$ Na$_2$MoO$_4$, 10 mol m$^{-3}$ NaCl and 0.01 mol m$^{-3}$ CoCl$_2$.

Measurement of carbohydrates

Samples for the extraction and measurement of carbohydrates were killed and extracted in boiling 80% (v/v) aqueous ethanol as described in Zeeman et al. (1998). Starch in the ethanol-insoluble fraction was determined as described by Hargreaves & ap Rees (1988). The ethanol-soluble fraction was dried in vacuo and the resultant material dissolved in 5 mL of water and stored at -20 °C. Sucrose, glucose and fructose in this fraction were determined enzymatically (Kunst, Dreager & Ziegenhorn 1984; Beutler 1984).

Measurement of photosynthesis and respiration

An ADC 225 Mk 3 Infra-Red Gas Analyser (ADC Bioscientific Ltd, Hoddesden, Herts, UK) was used to measure the rates of photosynthesis (CO$_2$ uptake) and respiration (CO$_2$ release) of hydroponically grown plants. Plants were grown in an apparatus in which the shoots and roots were held in compartments separated by an airtight seal. The roots were suspended in a mineral nutrient solution which was continuously aerated. After confirming the integrity of the seals, differential measurements of the CO$_2$ content of the air flowing in and out of these two chambers were made over the diurnal cycle.

$^{14}$CO$_2$ pulse-chase labelling of intact plants

Intact, soil-grown plants were introduced into an illuminated glass chamber. The light intensity was the same as that used to grow the plants (170 µmol quanta m$^{-2}$ s$^{-1}$). In the middle of the photoperiod the chamber was sealed and $^{14}$CO$_2$ released by the acidification of sodium $^{14}$C-bicarbonate. The concentration of CO$_2$ in the chamber was 0.3% (v/v), with a specific activity of 35 µCi mmol$^{-1}$. After 1 h the $^{14}$CO$_2$ was removed and air pumped through the chamber for the rest of the photoperiod. During the subsequent night the chamber was closed and a vial containing 1 mL of 10% (w/v) KOH was placed inside to collect $^{14}$CO$_2$ released from the plants. This KOH was replaced every hour.

To supply $^{14}$CO$_2$ to hydroponically grown plants, the apparatus described for the measurement of photosynthesis and respiration was used. The experiment was conducted essentially as described above for soil-grown plants. The root chamber was continuously aerated throughout the experiment. Air leaving the root chamber during the day passed through a series of three solid KOH traps to capture released $^{14}$CO$_2$. These traps were renewed at the beginning of the night, and a second set of traps attached to the outflow from the shoot chamber.

Analysis of the distribution of $^{14}$C

The amount of $^{14}$C present in different fractions of the plant was determined by liquid scintillation counting. Plants were harvested, killed and extracted in boiling 80% (v/v) ethanol. Ethanol-insoluble and soluble fractions were prepared as described above for the measurement of carbohydrates and in Zeeman et al. (1998). The total $^{14}$C in the insoluble material was determined by dissolving an aliquot of the material in Scintran Tissue Solubliser (BDH Chemicals Ltd, Poole, Dorset, UK). To measure the $^{14}$C in starch the starch was digested as described in Zeeman et al. (1998) and the liberated glucose separated by paper chromatography (Smith 1962).

The soluble fractions were fractionated further into neutral, acidic and basic compounds by ion exchange chromatography (Harley & Beevers 1963) using sequential columns of Dowex 50 W and Dowex 1 ion exchange resin. After the elution of basic compounds from the Dowex 50 W column, nucleotides were eluted according to Fowler & ap Rees (1970). The eluates were dried down in vacuo and resuspended in water. Sucrose, glucose and fructose in the neutral fraction were resolved by paper chromatography (Smith 1962). The recoveries of labelled metabolites after ion exchange and paper chromatography were consistently high (between 90% and 99% of the amount applied).

RESULTS

The accumulation of starch in the sex mutants

At the end of the day, leaves of mature sex4 plants contain almost three times as much starch as wild-type plants (Zeeman et al. 1998), whilst those of sex1 plants contain five times as much (Trethewey & ap Rees 1994). To investigate whether this high starch phenotype arises from the gradual accretion of starch during development, the starch content of leaves of different ages was measured. Four mature rosettes, each with 25–30 leaves, were harvested at the end of the day and each was divided into six fractions. The first
fraction contained the four youngest visible leaves. Fraction two contained the next three youngest leaves and fraction three, the following three youngest leaves, and so on. The last fraction (fraction 6) contained all the remaining, oldest leaves.

All the wild-type leaves contained between 6 and 10 mg starch g⁻¹ fresh weight (FW) (Fig. 1). Both the youngest leaves (fractions 1 and 2) and the very oldest leaves (fraction 6) contained slightly less starch than the most recently expanded leaves (fractions 3 and 4; $P \leq 0.05$). In contrast, the starch content of the leaves of both mutants increased appreciably with increasing age of the leaf (Fig. 1), showing that the high starch phenotype develops progressively as the leaves age. The starch content of the youngest leaves (fraction 1) of the sex⁴ mutant was similar to the wild type ($p \geq 0.05$), but all subsequent sex⁴ fractions contained significantly more starch than the wild-type fractions ($P \leq 0.01$). In sex¹, the high-starch phenotype was apparent in all the leaves, including the youngest leaves sampled ($P \leq 0.01$ with respect to the wild type). The mean starch contents, when all the leaves of each plant were considered together, were $7.14 \pm 0.35$, $22.82 \pm 0.77$, and $34.87 \pm 2.01$ for the wild type, sex⁴ and sex¹, respectively. These are close to the values found in earlier studies (Trethewey & ap Rees 1994; Zeeman et al. 1998).

**The effect of sex⁴ mutation on leaf sucrose and hexose content**

Transitory starch is degraded at night to provide substrates for sucrose synthesis. We investigated the effect of the sex⁴ mutation on the sucrose and hexose content of the leaves throughout the diurnal cycle. Leaves of wild-type and sex⁴ plants were harvested at three-hourly intervals and the sugars extracted.

During the day the sucrose contents of wild-type and sex⁴ leaves were similar, but during the night the leaves of sex⁴ plants contained less sucrose than wild-type leaves (Fig. 2a). Frequent measurements made during the first two hours of the night revealed that, at the onset of darkness, there was a fall in the sucrose content in both wild-type and mutant leaves (Fig. 2b). In the wild type this fall was transient and, after 2 h, the leaf sucrose content was restored to its daytime level. In the mutant leaves, the sucrose content was not restored after the initial fall and remained at approximately half of its daytime level.

There were appreciable differences in the free hexose content of the leaves of the mutant and wild type. At all times the glucose content exceeded the fructose content and the contents of the two sugars changed in a similar manner with respect to one another (Figs 2c & d). In the wild type this fall was transient and, after 2 h, the leaf sucrose content was restored to its daytime level. In the mutant leaves, the sucrose content was not restored after the initial fall and remained at approximately half of its daytime level.

The absolute rates of photosynthesis and respiration (measured with an infra-red gas analyser) varied slightly between experiments but the patterns were consistent. The rates of photosynthesis were similar in the wild type and the mutants (Fig. 3). However, there were consistent differences in rates and patterns of respiration between the wild type and the mutants. At the onset of the night, the rate of respiration in the shoots of the wild type, sex⁴ and sex¹ were similar. This rate of respiration was maintained by the wild-type leaves throughout the night, whereas in
both mutant lines, the rate of respiration fell within the first few hours (Fig. 3).

The pattern of root respiration throughout the diurnal cycle also differed between the wild type and the sex mutants (Fig. 3). In both mutants the rate of respiration fell shortly after the onset of darkness whereas that of the wild type did not. Conversely, both mutants showed a marked increase in the rate of root respiration during the first hours of the day. The differences in respiration between the sex1 mutant and the wild type were consistently greater in magnitude than between sex4 and the wild type.

The metabolism of $^{14}$CO$_2$ by sex4 and the wild type

Pulse-chase experiments were performed to discover how assimilated carbon is partitioned in wild-type and sex4 plants, and to investigate the extent of starch metabolism in sex. In these experiments, $^{14}$CO$_2$ was supplied to photosynthesizing plants for 1 h in the middle of the photoperiod (the pulse) to label the carbohydrate pools. The $^{14}$CO$_2$ was then replaced with air (the chase) and the plants allowed to continue photosynthesizing for the rest of the day. This chased label out of the pools of rapidly turned-over metabolites, such as sucrose, so that compounds derived from the $^{14}$C-labelled starch during the night could be detected. Plants were then incubated in the dark and metabolized their $^{14}$C-labelled starch, increasing the specific activity of compounds derived from the starch.

In each experiment, five samples were harvested, each consisting of the complete rosette of a single plant. The plants used in this experiment were grown in soil and root tissue was not harvested. Samples were taken immediately after the pulse (Pulse), at the end of the day (Chase 1) and 5·5, 7 and 12 h into the night (Chase 2, 3 and 4, respectively).

There were numerous differences in the distribution of $^{14}$C between sex4 plants and the wild type throughout the experiment. The distribution of $^{14}$C in the pulse samples indicated that, compared with the wild type, photosynthetic partitioning in the mutant was biased towards soluble products and away from starch. This was shown by the increase in label in soluble products (sugars and acidic compounds) and a decrease in the label in insoluble products (starch), relative to the wild type. Both of these differences between the mutant and the wild type were statistically significant ($P < 0.01$). This is consistent with earlier findings that sex4 plants synthesize less starch during a single day than the wild type (Zeeman et al. 1998).

The distribution of $^{14}$C in the chase samples indicates that the rate of starch degradation was lower in sex4 than in the wild type.

Figure 2. The sugar content of leaves of wild-type Arabidopsis (○, —) and the sex4 mutant (●, ⋯) during the day and night. Leaves were killed and extracted in boiling 80% (v/v) ethanol and the sucrose (a and b), glucose (c) and fructose (d) in the ethanol-soluble fraction were measured. In (a) (c) and (d), each point is the mean of measurements on four replicate samples. Each sample was composed of all the leaves of a single plant and error bars represent the SEM. In some cases the error bars are smaller than the symbols. In (b), all the leaves of four plants were harvested and treated as a single sample.
Insoluble compounds 66·3 ± 1·9

Intact soil-grown plants were exposed to 14CO2 for 1 h in the middle of the photoperiod. The 14CO2 was removed and a sample harvested.

Soluble compounds 34·0 ± 1·9

The distribution of assimilated 14C in different fractions of wild-type leaves after a pulse of 14CO2 and a 17 h chase period. Table 1.

Carbohydrate metabolism in sex mutants of Arabidopsis

Figure 3. Photosynthesis and respiration of the shoots (top panel) and respiration of the roots (bottom panel) of wild-type Arabidopsis (dashed line), and the mutant lines sex1 (solid line) and sex4 (dotted line). Continuous measurements of CO2 uptake or emission were made using an infra-red gas analyser. Positive values represent CO2 uptake, negative values represent CO2 emission. The insert in the top panel shows the changes in shoot respiration in the first four hours of the night in greater detail.

Wild type. During the night essentially all the 14C was lost from starch in the wild type, whereas in sex4 only 60% of the labelled starch was degraded. Furthermore, in the wild type the greatest loss of label was during the first 5·5 h of the night, whereas in sex4 it was in the last 5 h.

In the wild type, sucrose was synthesized from the 14C released from starch. At the end of the day there was very little label in sucrose (Table 1, ‘Chase 1’ sample), but as 14C-starch was degraded, so 14C-sucrose appeared (‘Chase 2’ sample). Loss of 14C from no other compound was large enough to account for the appearance of label in sucrose. During the same period (0–5·5 h into the night), there was also a rise in 14C-sucrose in the mutant, and a decrease in 14C-starch sufficient to account for it (Table 2). However, the rise in 14C-sucrose was smaller than in the wild type and the 14C was not necessarily derived from the degradation of 14C-starch. First, there was an appreciable decrease in 14C-glucose in the mutant which could have accounted for the appearance of the 14C in sucrose. Secondly, during the last 5 h of the night, there was an appreciable fall in the label in sucrose, despite the continued degradation of 14C-starch. Thus it seems likely that the initial increase in 14C-sucrose was due in part to the transfer of label from glucose, and that the movement of carbon from starch to sucrose was considerably slower in sex4 than in the wild type.

The rate of 14CO2 release from wild-type plants rose during the night and peaked after 8 h of darkness. The peak in 14C-sucrose occurred earlier, suggesting that the 14CO2 may have been generated primarily by the respiration of the 14C-sucrose in the sink tissues of the plant, after translocation via the phloem. In sex4 this peak 14CO2 production occurred about 10 h into the night, again when the propor-

Table 1. The distribution of assimilated 14C in different fractions of wild-type leaves after a pulse of 14CO2 and a 17 h chase period.

Intact soil-grown plants were exposed to 14CO2 for 1 h in the middle of the photoperiod. The 14CO2 was removed and a sample harvested immediately (‘Pulse’). Further ‘Chase’ samples were harvested during the ensuing incubation in air, after 5 h (end of day), 10·5 h (5·5 h into night) and 17 h (end of night). Each sample was composed of the complete rosette of a single plant. The tissue was killed and extracted in boiling 80% (v/v) ethanol, and the soluble and insoluble fractions fractionated as described in Materials and Methods. The 14CO2 released from the plants during the night was collected in a KOH trap. The 14C in each fraction was determined by liquid scintillation counting. The experiment was replicated three times, each time with a different batch of plants, and the values given are the means ± SEM.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pulse (after 1 h 14CO2)</th>
<th>Chase 1 (end of day)</th>
<th>Chase 2 (5·5 h into night)</th>
<th>Chase 3 (7 h into night)</th>
<th>Chase 4 (end of night)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble compounds</td>
<td>34·0 ± 0·4</td>
<td>22·7 ± 1·7</td>
<td>34·9 ± 1·9</td>
<td>33·0 ± 2·2</td>
<td>28·1 ± 2·5</td>
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<tr>
<td>Neutral compounds</td>
<td>15·5 ± 0·5</td>
<td>2·8 ± 0·6</td>
<td>11·5 ± 0·6</td>
<td>8·0 ± 1·1</td>
<td>2·8 ± 0·3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10·0 ± 1·6</td>
<td>0·6 ± 0·3</td>
<td>6·9 ± 2·0</td>
<td>4·3 ± 1·1</td>
<td>0·9 ± 0·3</td>
</tr>
<tr>
<td>Glucose</td>
<td>1·0 ± 0·2</td>
<td>0·8 ± 0·3</td>
<td>0·9 ± 0·2</td>
<td>0·7 ± 0·2</td>
<td>0·9 ± 0·3</td>
</tr>
<tr>
<td>Fructose</td>
<td>0·8 ± 0·1</td>
<td>0·1 ± 0·0</td>
<td>0·4 ± 0·0</td>
<td>0·3 ± 0·1</td>
<td>0·1 ± 0·0</td>
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<tr>
<td>Acidic compounds</td>
<td>11·4 ± 0·2</td>
<td>15·0 ± 1·0</td>
<td>15·6 ± 1·0</td>
<td>16·2 ± 0·9</td>
<td>15·4 ± 1·9</td>
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<td>Basic compounds</td>
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<td>4·8 ± 0·2</td>
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<tr>
<td>Nucleotides</td>
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<td>1·0 ± 0·2</td>
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<td>Insoluble compounds</td>
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<td>77·3 ± 1·7</td>
<td>63·2 ± 1·5</td>
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<td>Starch</td>
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<td>10·7 ± 1·8</td>
<td>1·1 ± 0·3</td>
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<td>Other insoluble material</td>
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<td>44·4 ± 3·1</td>
<td>52·5 ± 3·5</td>
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<td>14CO2</td>
<td>–</td>
<td>–</td>
<td>1·9 ± 0·1</td>
<td>3·8 ± 0·3</td>
<td>10·2 ± 0·4</td>
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</table>

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tion of label in sucrose was falling. The total label released as $^{14}$CO$_2$ by the mutant was less than by the wild type.

The export of $^{14}$C-labelled photoassimilates to the roots

To determine how much $^{14}$C was exported to the roots during the day and night, another pulse-chase experiment was performed, which allowed the roots and shoots to be analysed separately. Plants were grown hydroponically in the apparatus described for the measurement of photosynthesis and respiration, with the shoots and roots held in separate chambers. The shoots were exposed to $^{14}$CO$_2$ for 1 h in the middle of the photoperiod and then allowed to continue photosynthesizing in air for the rest of the day. Samples of wild-type and $sex4$ plants were harvested at the end of the day and the end of the subsequent night. Each sample consisted of two plants which were divided into roots and shoots. The distribution of $^{14}$C in the plants was then determined (Table 3).

In the wild-type plants, 93% of the label assimilated in the pulse was still in the shoots at the end of the day and only 7% had been exported to the roots. However, in the mutant the percentage of label exported to the roots during the same period was 2.5-fold greater (17%). Interestingly, during the night the reverse was true; the wild-type plants continued to export $^{14}$C-labelled compounds, and by the end of the night a total of 23% of the carbon assimilated during the pulse had been exported to the roots. In the mutant, the rate of export of label during the night was much lower than during the day, yet by the end of the night both lines had exported roughly the same proportion of $^{14}$C to the roots in total.

DISCUSSION

Our results show that the endoamylase-deficient mutant $sex4$ is appreciably different from the wild type in terms of its photosynthetic partitioning, assimilate export and patterns of respiration. When considered together, the data allow a picture of whole-plant carbohydrate fluxes to be compiled, and enable an interpretation of the differences between the wild type and the mutant.

Carbohydrate fluxes in the wild type

In the wild type, approximately half the carbon that is assimilated during the day is stored in the leaves as starch.

Table 2. The distribution of assimilated $^{14}$C in different fractions of $sex4$ leaves after a pulse of $^{14}$CO$_2$ and a 17-h chase period. The details of the experiment are described in the legend to Table 1

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pulse (after 1 h $^{14}$CO$_2$)</th>
<th>Chase 1 (end of day)</th>
<th>Chase 2 (5.5 h into night)</th>
<th>Chase 3 (7 h into night)</th>
<th>Chase 4 (end of night)</th>
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<td>Soluble compounds</td>
<td>$43.8 \pm 1.9$</td>
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<td>$6.8 \pm 0.5$</td>
<td>$4.7 \pm 0.6$</td>
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<tr>
<td>Sucrose</td>
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<td>$3.6 \pm 0.4$</td>
<td>$4.5 \pm 0.5$</td>
<td>$1.8 \pm 0.8$</td>
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<td>Glucose</td>
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<td>$^{14}$CO$_2$</td>
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<td>–</td>
<td>$1.3 \pm 0.1$</td>
<td>$1.8 \pm 0.1$</td>
<td>$7.2 \pm 0.3$</td>
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</table>

Figure 4. The release of $^{14}$CO$_2$ during the night from wild-type Arabidopsis (open bars) and $sex4$ plants (filled bars) during the degradation $^{14}$C-labelled starch. Plants were exposed to a 1 h pulse of $^{14}$CO$_2$ during the middle of the photoperiod, then incubated for the rest of the photoperiod and the subsequent night in air. A vial containing 1 mL of 10% KOH (w/v) was renewed at hourly intervals during the night. The $^{14}$CO$_2$ absorbed by the KOH trap was measured. Each bar is the mean (± SEM) of measurements made in three independent experiments. In some cases the error bars are too small to be plotted.
Carbohydrate metabolism in sex mutants of Arabidopsis

To the large decrease in 14C in starch (Table 1, samples ‘Chase 2’ and ‘Chase 3’), while there is a steady degradation, label appears transiently in the sucrose pool at more than 1 mg h⁻¹ relative to the rate of starch degradation. Starch is mobilized at more than 1 mg h⁻¹ relative to the rate of starch degradation. This is indicated by the sucrose (and hexose) content of the leaves as they age (Fig. 1). This also occurs in the sex1 mutant, which is unable to export glucose from the chloroplast during starch degradation (Trethewey & ap Rees 1994). In this case, the starch excess phenotype is established earlier, which is consistent with the greater severity of the phenotype in sex1, compared with sex4. (Trethewey & ap Rees 1994; Zeeman et al. 1998)

The movement of carbon from starch to sucrose, and then into ethanol-insoluble material (such as cell wall components and proteins) is demonstrated by the 14C-labelling experiment summarized in Table 1. During 14C-starch degradation, label appears transiently in the sucrose pool (samples ‘Chase 2’ and ‘Chase 3’), while there is a steady increase in 14C in the ethanol-insoluble material throughout the night. The flux of carbohydrate through sucrose is rapid. This is indicated by the small size of the sucrose pool relative to the rate of starch degradation. Starch is mobilized at more than 1 mg h⁻¹ g⁻¹ FW (Zeeman et al. 1998), and it is likely that a large fraction of this will be converted to sucrose. The sucrose pool is approximately 0.5 mg g⁻¹ FW (Fig. 2a). Therefore, if only half of the starch is converted to sucrose, this would still represent 12 times the sucrose pool over the course of the night. This high turnover is also reflected by the small increase in the 14C in sucrose relative to the large decrease in 14C in starch (Table 1, samples ‘Chase 2’ and ‘Chase 3’).

Direct effects of the sex4 mutation

The reduction in chloroplastic endoamylase in sex4 has several direct effects on carbohydrate metabolism during the night. The rate of starch degradation is reduced to about half that in the wild type, leading to an imbalance between starch synthesis and degradation (Table 1 and Zeeman et al. 1998). As a result, the starch content increases gradually in the leaves as they age (Fig. 1). This also occurs in the sex1 mutant, which is unable to export glucose from the chloroplast during starch degradation (Trethewey & ap Rees 1994). In this case, the starch excess phenotype is established earlier, which is consistent with the greater severity of the phenotype in sex1, compared with sex4. (Trethewey & ap Rees 1994; Zeeman et al. 1998)

The restriction of starch degradation in sex4 results in a reduction in sucrose synthesis compared with the wild type. This is indicated by the sucrose (and hexose) content of the leaves in the night (Fig. 2) and by the movement of 14C in the labelling experiments (Tables 1 & 2). First, the fall in the sucrose content of wild-type leaves at the onset of the night is transient, whereas in the mutant the sucrose content falls and remains low. This suggests that the rate of sucrose synthesis is reduced due to a limitation in the availability of substrates derived from starch degradation. This hypothesis is supported by the pattern of movement of 14C out of starch and into sucrose in the mutant; although there is an increase in 14C in sucrose in sex4 leaves, the increase is less than in the wild type and does not correlate well with the movement of 14C out of starch. Furthermore, some of the 14C in sucrose may have been derived from 14C-glucose present in the leaf rather than from 14C-starch (Table 2, samples ‘Chase 1’ and ‘Chase 2’). Secondly, the lower rate of export of 14C from the shoots to the roots during the night (6·9 and 15·8% of the total 14C assimilated in the mutant and wild type, respectively) is consistent with a lower rate of sucrose synthesis, as it is likely that most export of 14C would be in the form of sucrose.

Table 3. The distribution of assimilated 14C in the shoots and roots of wild-type and sex4 plants. An experiment similar to that described in the legend of Table 1 was carried out using hydroponically grown plants. The experiment was performed in an apparatus that allowed the roots and shoots to be kept in different compartments with an airtight seal between them. Two samples were harvested, one at the end of the night and one at the end of the following night. Each sample was composed of two complete plants, and was divided into shoots and roots. Samples were extracted and fractionated as described in Materials and Methods.
Respiration in sex4 is also affected by the reduction in starch degradation. The rate of CO₂ production of both the shoots and the roots of the mutant declines during the dark, relative to the wild type. In both cases there are two possible explanations. Respiration may be substrate limited; in the leaves this could result directly from the reduced abundance of starch degradation products, whereas in the roots, reduced export of sucrose from the leaves could have a similar effect. Alternatively, the requirement for respiratory ATP production could be reduced. In leaves, a reduction in the rate of sucrose synthesis and export would be accompanied by a reduction in ATP consumption. Similarly a reduction in growth of the roots would reduce the requirement for ATP. These explanations are not mutually exclusive and both may be true to an extent.

**Indirect effects of the sex4 mutation**

Our results provide considerable evidence that, in the sex4 mutant, there is a shift in photosynthetic partitioning away from starch in favour of sugars. First, sex4 synthesizes less starch during the day than the wild type, despite a similar rate of photosynthesis (Fig. 3 and Zeeman et al. 1998). Secondly, the 14C-labelling experiments show that the proportion of assimilated CO₂ partitioned into starch is less in the mutant than in the wild type, whereas that partitioned into sugars is greater (Table 1 and 2). Thirdly, whilst the sucrose content during the day is similar in the wild type and the mutant (Fig. 2a), the rate of export of label to the roots in the mutant is more than two times higher than in the wild type (16·9 and 6·9%, respectively, Table 3).

The changes in the metabolism of the sex4 mutant partly compensate for its reduced ability to mobilize transitory starch during the night. As a result of the alterations in partitioning and assimilate export, the amount of assimilated carbon reaching the roots is similar to that in the wild type. In fact, despite the considerable differences in the distribution of 14C in the wild type and sex4 samples taken early in the 14C-labelling experiments, the distribution of 14C in the samples taken at the end of the night was remarkably similar in the two lines. The most notable difference between these samples was the amount of label remaining in starch; in the wild type it was 1%, whilst in the mutant it was 21%. This means that the amount of carbon that has been mobilized from starch to support the synthesis of new tissue is lower in the mutant than in the wild type, explaining the slow overall growth of sex4 (Zeeman et al. 1998).

It has been shown for other species that alterations in the synthesis of transitory starch can lead to compensatory changes in sucrose synthesis and export. In the leaves of transgenic potato plants, where starch synthesis is restricted by reducing ADP-glucose pyrophosphorylase activity (Müller-Röber, Sonnewald & Willmitzer 1992) the synthesis and export of sucrose is increased during the day and reduced at night (Leidreiter et al. 1995), compared with wild-type plants. Similarly, the rate of export from the leaves of a starchless tobacco mutant during the day is higher than in the wild type (Huber & Hanson 1992). It seems likely that the increase in sucrose synthesis and export in these cases is caused by a feed-forward effect due to the accumulation of photosynthetic intermediates.

The nature of the alterations in carbohydrate metabolism in sex4 is distinct from those reported for mutants and transgenic plants in other species. Whereas in the examples cited above, a shift towards sucrose synthesis results from a disruption of starch synthesis, in sex4, the shift in partitioning toward sucrose synthesis occurs without any disruption in the pathway of starch synthesis. Therefore, we argue that the increased synthesis of sucrose does not occur due to a feed-forward effect from the accumulation of intermediates. It is not clear how the change in photosynthetic partitioning in sex4 is brought about. We propose that either the increase in starch content of the leaves, or more likely, the changes in sugar content of the leaves feed into signalling pathways which result in altered expression of genes encoding enzymes involved in the synthesis of sucrose and starch. The observation that both sex4 and sex1 mutants have a reduced soluble starch synthase activity (Caspar et al. 1991; Zeeman et al. 1998) is consistent with this hypothesis.

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**REFERENCES**


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