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Modification of Carbon Partitioning, Photosynthetic Capacity, and O_2 Sensitivity in Arabidopsis Plants with Low ADP-Glucose Pyrophosphorylase Activity[1]

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Wild-type Arabidopsis plants, the starch-deficient mutant TL46, and the near-starchless mutant TL25 were evaluated by noninvasive in situ methods for their capacity for net CO_2 assimilation, true rates of photosynthetic O_2 evolution (determined from chlorophyll fluorescence measurements of photosystem II), partitioning of photosynthate into sucrose and starch, and plant growth. Compared with wild-type plants, the starch mutants showed reduced photosynthetic capacity, with the largest reduction occurring in mutant TL25 subjected to high light and increased CO_2 partial pressure. The extent of stimulation of CO_2 assimilation by increasing CO_2 or by reducing O_2 partial pressure was significantly less for the starch mutants than for wild-type plants. Under high light and moderate to high levels of CO_2, the rates of CO_2 assimilation and O_2 evolution and the percentage inhibition of photosynthesis by low O_2 were higher for the wild type than for the mutants. The relative rates of $^{14}CO_2$ incorporation into starch under high light and high CO_2 followed the patterns of photosynthetic capacity, with TL46 showing 31% to 40% of the starch-labeling rates of the wild type and TL25 showing less than 14% incorporation. Overall, there were significant correlations between the rates of starch synthesis and CO_2 assimilation and between the rates of starch synthesis and cumulative leaf area. These results indicate that leaf starch plays an important role as a transient reserve, the synthesis of which can ameliorate any potential reduction in photosynthesis caused by feedback regulation.

Because plant productivity is governed by photosynthetic activity and sink activity for utilizing photosynthate (see Zamski and Schaffer, 1996), it is important to understand the environmental and genetic factors affecting these processes. In general, photosynthesis is limited mainly by light harvesting and assimilatory power under low light and by carboxylation and photorespiration under low CO_2. Under saturating light and CO_2, however, photosynthesis may be controlled by processes that convert triose-P into starch and Suc (Sage, 1990, 1994; Stitt, 1996). Thus, the capacity to utilize triose-P for carbohydrate synthesis can establish an upper limit for the maximum rate of photosynthesis under CO_2- and light-saturated conditions (Sage, 1990; Sharkey et al., 1995). This is clearly demonstrated under certain conditions by the response of photosynthesis of C_3 plants to low O_2. In many instances, the increase in CO_2 assimilation attributable to the reduction in photorespiration under low O_2 can be predicted accurately based on the known kinetic properties of Rubisco. However, when the extent of stimulation of photosynthesis by C_3 plants under subatmospheric O_2 is less than predicted, or when there is reversed O_2 sensitivity, photosynthesis is considered to be feedback limited as a result of restrictions on triose-P utilization (Sharkey, 1985; Leegood and Furbank, 1986; Sage and Sharkey, 1987; Hanson, 1990; Sun et al., 1997). Limitations on triose-P utilization have also been suggested to be responsible for the low CO_2 saturation response and decreased electron transport under high light (Sharkey et al., 1988; Peterson and Hanson, 1991; Eichelmann and Laisk, 1994).

The bulk of the photosynthetically fixed carbon in mature leaves is partitioned between Suc and starch. Based on experimental results and biochemical models, the events controlling the partitioning of photosynthate between Suc and starch synthesis occur in the cytoplasm (Eichelmann and Laisk, 1994; Stitt, 1996). Hence, triose-P may be converted preferentially into Suc at lower rates of triose-P production, with increased partitioning to starch synthesis occurring as Suc synthesis reaches saturation. Alternatively, carbon partitioning may be programmed so that a portion of the photosynthate is allocated for starch synthesis.

Efforts have been made to increase Suc production by the manipulation of Suc-P synthase activity, which catalyzes one of the key regulatory steps in the cytoplasm. Elevation of this enzyme activity by overexpressing the maize Suc-P synthase in tomato leads to an increase in Suc synthesis and in the rate of photosynthesis at high CO_2 and high light (Galtier et al., 1993, 1995; Micallef et al., 1995). However, under some circumstances, the capacity for Suc synthesis

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Abbreviations: A, CO_2 assimilation rate; AGPase, ADP-Glc pyrophosphorylase; F_m', maximal yield of fluorescence from a saturating flash of white light; F_s, steady-state fluorescence; J_{O2}, gross rate of O_2 evolution; 3-PGA, 3-phosphoglycerate; Φ_{PSII}, quantum yield of PSII.

may be restricted by limitations on phloem loading, transport, or unloading. Some transport studies have shown that the export rate of photosynthate from leaves does not increase when plants are shifted to a higher irradiance (Silvius et al., 1979) or to a CO_2-enriched environment (Ho, 1977; Huber et al., 1984).

A portion of the fixed carbon is also allocated to formation of starch in many plants. AGPase is an important regulatory enzyme controlling starch biosynthesis. Mutations reducing the activity of the enzyme lead to starch deficiency, as demonstrated in Arabidopsis leaf *adg2* (TL46) and *adg1* (TL25) mutants (Lin et al., 1988a, 1988b) and maize endosperm *shrunken-2* and *brittle-2* mutants (Tsai and Nelson, 1966; Dickinson and Preiss, 1969). Neuhaus and Stitt (1990) reported that a reduction in AGPase activity in leaves of the Arabidopsis mutant TL46 resulted in a reduction in starch synthesis, but with differential effects on Suc biosynthesis and photosynthesis, depending on the light intensity. Under low-light conditions fixed carbon was partitioned mainly into Suc, with no significant effect on photosynthesis between the mutant and the wild type. Under high light, however, Suc synthesis, as well as starch synthesis and photosynthesis, were inhibited in the mutant. These studies were conducted with excised leaf tissue in an O_2 electrode chamber under saturating CO_2, conditions that are unnatural compared with those in situ.

In the present study a new approach was used to investigate the photosynthetic properties of wild-type Arabidopsis and two starch mutants, a starch-deficient mutant, TL46, and a near-starchless mutant, TL25, using a special plant chamber/gas-exchange system that is capable of measuring A and Φ_{PSII} values by fluorescence measurements on intact plants (Donahue et al., 1997). With this system, $^{14}CO_2$ labeling (and rates of partitioning into starch and Suc), A, and PSII activity can be determined simultaneously under varying levels of CO_2 and O_2. We present evidence using noninvasive methods that partitioning of fixed carbon into starch plays a far more prominent role in dictating the overall photosynthetic potential than previously thought. Our finding suggests that leaf starch serves as a transient reserve that can accommodate relatively large increases in triose-P production by the C_3 pathway, thereby preventing potential feedback of this primary assimilatory process.

MATERIALS AND METHODS

Plant Growth

Plant materials used in this study were Arabidopsis Heynh cv Columbia wild type, TL46, and TL25. These lines were obtained from the Arabidopsis Repository at Ohio State University (Columbus). The mutant TL46 contains a missense mutation of the *adg2* gene, which codes the large subunit structural gene of AGPase (Wang et al., 1997). The mutant TL25 contains a mutation of the *adg1* gene, which codes the small subunit structural gene of AGPase (Lin et al., 1988b). Plants were grown in controlled environmental growth chambers with a 12-h photoperiod and a PPFD of either 100 (low light) or 350 (high light) μmol m^{-2} s^{-1} provided by fluorescent lights. Day and night temperatures were 24°C ± 1°C and 18°C ± 1°C, respectively. RH in the growth chambers was maintained above 70%.

For photosynthetic studies, the Arabidopsis plants were planted in a soil mixture containing 60% peat, 20% pumice, 20% sand, and 4.8 kg m^{-3} agricultural lime in 50-mL polypropylene centrifuge tubes (model 25325-50, Corning Inc., Corning, NY). The tubes were painted a dark color to deter algae growth in the soil and perforated at the bottom for water drainage. After 1 to 2 weeks, plants were thinned to one plant per tube. Plants were irrigated daily with Hoagland solution (Hoagland, 1950). Healthy, 5- to 6-week-old plants were used for experiments. Plants were used at the beginning of the bolting stage so that the wild-type and mutant plants were at similar developmental stages.

Enzyme Extraction and Assay

Arabidopsis leaves were collected about 2 h into the light period and stored in liquid nitrogen until analysis. The leaves were extracted and analyzed on the day they were sampled. The leaves were extracted in buffer solution containing 50 mM Mops-NaOH, pH 7.5, 15 mM $MgCl_2$, 1 mM EDTA, 0.1% Triton X-100, 2.5 mM DTT, and 2.5% glycerol. The homogenate was centrifuged in a microcentrifuge (model 235, Fisher Scientific) at maximum speed (approximately 12,000*g*) for 3 min at 4°C. The supernatant was desalted through a small Sephadex G-25 (superfine) column before assaying for AGPase activity, according to the method of Sowokinos (1976). AGPase activities of the desalted leaf extracts were assayed at 37°C in a reaction mixture containing 80 mM glycylglycine, pH 7.5, 5 mM $MgCl_2$, 10 mM NaF, 2.5 mM DTT, 0.5 mM NADP, 1 unit of phosphoglucomutase, 1 unit of Glc-6-P dehydrogenase, 2 mM ADP-Glc, 4 mM 3-PGA, and 1.5 mM sodium pyrophosphate. The absorption of the formed NADPH at 340 nm (absorption coefficient, 6.22 mM^{-1}) was recorded without the addition of sodium pyrophosphate (control) and with the addition of sodium pyrophosphate in a Perkin-Elmer 552A spectrophotometer. Protein content was determined using the Bradford procedure with BSA as the standard (Bradford, 1976).

Gas Exchange

Rates of CO_2 assimilation on whole plants were measured with a Bi-2-dp mini cuvette controller (Bingham, Hyde Park, UT), an MK3–225 IR gas analyzer (ADC, Hoddesdon, Hertfordshire, UK), and data were obtained with a linear chart recorder (Tekmar, Cincinnati, OH). Gas exchange was measured by CO_2 depletion in the differential mode with an open system in which a given gas mixture flowed through the reference cell and the sample cell (in line with the plant enclosed in a cuvette). The threaded tubes in which plants were grown were inserted into a threaded port in the bottom of the special laboratory-built leaf chamber (Donahue et al., 1997) and sealed with modeling clay. The gas-flow rate in the leaf chamber was 1 L min^{-1}. CO_2 depletion in the leaf chamber was about 10% to

15% under high light. The plant cuvette contained a copper-constantan thermocouple, which was placed in contact with the lower epidermis of a leaf to monitor plant temperature. Water vapor leaving the chamber was measured with a digital hygrometer (Fisher Scientific). The leaf chamber had a temperature-controlled water jacket connected to a water bath. PPFD was measured with a quantum sensor (model 185, Li-Cor, Lincoln, NE). RH was maintained at 60% to 80% in the leaf chamber. Some control tests were made on gas exchange after the aerial portion of the plant was removed, and showed that the CO_2 exchange from roots and soil was negligible. The soil surface area was small (diameter of tube, 1 inch) and the bottom hole in the tube was sealed by modeling clay during the assay.

Chlorophyll Fluorescence

Chlorophyll fluorescence was measured with a PAM 101 fluorometer (Heinz-Walz, Effeltrich, Germany) simultaneously with gas-exchange measurements, while Arabidopsis plants were enclosed in the gas-exchange chamber, as described above. The fiber-optic bundle of the fluorometer was positioned on the top corner of the leaf chamber at an inclined angle (45°) to minimize shading of the plant from the actinic light source. The distance between the fluorometer sensor and the plant was about 4 cm. The plant area, which was covered by the saturating pulse of light and from which the fluorescence signal was received by the sensor, was about 6 cm^2 (covering approximately 30% of the plant canopy). F_s was monitored continuously, and for periodic determination of F_m', saturating pulses (1-s duration) of white light (10,000 μmol m^{-2} s^{-1}) were applied by a PAM 103 trigger-control unit (Heinz-Walz). Φ_{PSII} was calculated as $(F_m' - F_s)/F_m'$, as described by Genty et al. (1989). J_{O2} was calculated as $(\Phi_{PSII} \times I_a \times F)/4$ (Genty et al., 1989; Edwards and Baker, 1993), where I_a, the light absorbed, was assumed to be 0.8 × PPFD, and F, a factor for the partitioning of photons between incident PSII and PSI, was assumed to be 0.5 (Donahue et al., 1997).

$^{14}CO_2$ Feeding and Assays of Starch and Suc Synthesis

$^{14}CO_2$ released by acidifying a $NaH^{14}CO_3$ solution was collected in a vacuumed, steel gas cylinder and then a specific composition of gases was added to acquire the desired concentration of $^{14}CO_2$ for feeding. An intact Arabidopsis plant grown in a 50-mL polypropylene tube was enclosed in the leaf chamber (see "Gas Exchange") and illuminated to obtain steady-state photosynthesis. The plants were then exposed to $^{14}CO_2$ gas (specific activity, 0.1 Ci mol^{-1}) for 10 min and chased for 10 min for measurement of partitioning into starch and Suc. A 1-min chase was also tested; the 10-min chase gave similar trends but less in the ionic fraction and more in starch and Suc without significant export from the leaf. A test for degree of retention of label in leaves showed that $^{14}CO_2$ partitioning into roots and stems was about 5% of the total $^{14}CO_2$ incorporation after a 10-min pulse and a 10-min chase (data not shown). Leaves were extracted several times by addition of hot 80% ethanol until the extract was colorless, and separated into soluble and insoluble fractions (Angelov et al., 1993). The ethanol-soluble fractions from each sample were pooled, dried under dry air at 50°C, resolubilized in 1.5 mL of distilled water, and frozen until analysis. The soluble fraction was passed through a Dowex 50 H^+ column and then through a Dowex 1 Cl^- resin column. The soluble neutral fraction eluted from the two columns by water was taken as the Suc fraction. Amino acids were eluted from the Dowex 50 H^+ column by 5 M NH_4OH, and phosphorylated intermediates were eluted from the Dowex 1 Cl^- column by 2 M HCl (accounting for 10%–30% of the total $^{14}CO_2$ fixed; partitioning into these fractions not shown). The residue (insoluble fraction) was homogenized with 1 mL of water to determine the extent of ^{14}C incorporation into starch. The radioactivity in each compound was determined with a liquid-scintillation counter (model LS7000, Beckman).

Leaf Area

Preliminary tests showed that the total leaf area of the plant determined by summation of the detached individual leaves was about 5% greater than the plant leaf area determined on intact rosettes. This was similar among the different genotypes and the 5% difference represents the degree of self-shading. In this study the plant leaf area was determined from a photocopy of the intact rosette using a computerized NIH image system (National Institutes of Health, Bethesda, MD) or a Li-3000 leaf area meter (Li-Cor).

RESULTS

AGPase Activity

AGPase activities of the wild type and the TL46 and TL25 mutants grown at high light and low light were determined (Table I). AGPase kinetics exhibited two phases of activity: an initial slope, which lasted for 1 to 2 min, followed by a lower steady-state slope, which was linear for at least 15 min. There was a similar reduction in AGPase activity in both low-light- and high-light-grown TL46, with the initial activity being 29% to 30% of that in the wild type and the steady-state activity being about 11% of that in the wild type, respectively. There was lower activity of AGPase in the mutant TL25, with initial activity

Table I. *AGPase activities in the leaves of the wild type and TL46 and TL25 mutants grown at low light (LL) (100 μmol m^{-2} s^{-1}) or high light (HL) (350 μmol m^{-2} s^{-1})*

For replication, $n = 2$. Values are given as ±SE.

Plant	Growth Light	AGPase Activity	
		Initial	Steady state
		μmol h^{-1} g^{-1} fresh wt	
Wild type	LL	46 ± 4	35 ± 3
	HL	58 ± 2	40 ± 2
TL46	LL	14 ± 1	4 ± 1
	HL	17 ± 2	5 ± 2
TL25	LL	9 ± 1	<1
	HL	9 ± 1	4 ± 1

in both low-light- and high-light-grown plants being 16% to 19% of that in the wild type. The steady-state activity in TL25 in low-light-grown plants was less than 3% of that in the wild type, whereas the value of high-light-grown TL25 plants was about 10% of that in the wild type. In general, the degree of reduction of AGPase activities in mutants compared with the wild type was similar to that previously reported for line TL46 (Lin et al., 1988a; Neuhaus and Stitt, 1990) and line TL25 (Lin et al., 1988b).

CO_2 Assimilation

The rates of CO_2 assimilation under various PPFDs and CO_2 partial pressures were studied in intact wild-type plants and AGPase mutants enclosed in a special chamber (Fig. 1). Whereas there was little or no difference in *A* between the wild type and the starch mutants under a lower PPFD and at atmospheric CO_2 (31 Pa), there were larger differences under saturating PPFD and CO_2 levels. At a PPFD of 800 μmol m^{-2} s^{-1} and a CO_2 partial pressure of 80 Pa, *A* was significantly higher in the wild type than in line TL46 (60% of that in the wild type), which in turn was higher than in line TL25 (50% of that in the wild type) (Fig. 1). Under these conditions plants grown in high light showed higher *A* than low-light-grown plants.

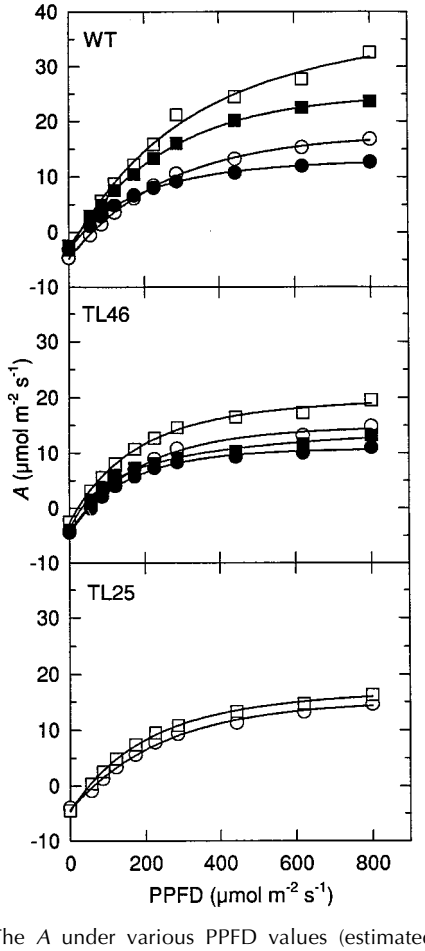

Figure 1. The *A* under various PPFD values (estimated absorbed values by multiplying incident light times 0.8) and CO_2 partial pressures in Arabidopsis wild type (WT), TL46, and TL25. The plants were grown under a PPFD of either 100 (●, ■) or 350 (○, □) μmol m^{-2} s^{-1}. CO_2 assimilation was conducted under various PPFD regimes at 25°C, an O_2 partial pressure of 20 kPa, and a CO_2 partial pressure of either 31 Pa (○, ●) or 80 Pa (□, ■). Symbols represent means of measurements of two plants. No experiments were conducted with low-light-grown TL25. SD values are omitted for clarity. The average SD was 0.6 μmol m^{-2} s^{-1}.

Similarly, these Arabidopsis lines showed different responses when subjected to higher CO_2 partial pressures. When the CO_2 partial pressure was shifted from 31 to 80 Pa, wild-type plants had significantly higher *A*, with the high-light-grown plants exhibiting a 94% increase and the low-light-grown plants showing a somewhat smaller increase of 86%. CO_2 assimilation rates were also stimulated by CO_2 enrichment in high-light-grown TL46 plants, but only by about one-third as much as in wild-type plants. TL46 plants grown under low light showed very little increase in *A* at the elevated CO_2 partial pressures. In high-light-grown TL25, *A* was saturated at atmospheric CO_2 levels; an increase in CO_2 partial pressure from 31 to 80 Pa did not significantly increase *A* (Fig. 1).

The starch mutants also showed differences in their response to light saturation (Fig. 1). In wild-type plants at 80 Pa CO_2, *A* continued to increase up to 800 μmol m^{-2} s^{-1} PPFD, whereas in lines TL46 and TL25, rates were near saturation at about 400 μmol m^{-2} s^{-1} PPFD.

Electron Transport

Electron-transport rates were measured simultaneously with *A*. Figure 2 shows that the J_{O2} values, as determined from chlorophyll fluorescence measurements of PSII yield, correlated very well with *A*. The rates of CO_2 assimilation at a given J_{O2} were higher under 80 Pa CO_2 than under 31 Pa CO_2 for both high- and low-light-grown wild-type plants. This pattern was expected, because high CO_2 suppresses photorespiration and increases the A/J_{O2} ratio. In TL46, *A* at a given J_{O2} was higher under 80 Pa CO_2 than under 31 Pa CO_2 in high-light-grown plants but not in low-light-grown plants. In high-light-grown TL25, however, *A* at a given J_{O2} was essentially identical at 80 and 31 Pa CO_2. Overall, these results indicate that modification of starch metabolism significantly affects *A* and electron transport through feedback regulation (see below).

The increase in J_{O2} with increasing PPFD in high-light-grown wild-type, TL46, and TL25 plants at 80 Pa CO_2 is shown in Figure 3. J_{O2} values were similar for the wild-type, TL46, and TL25 plants below a PPFD of 300 μmol m^{-2} s^{-1}. Above 300 μmol m^{-2} s^{-1}, however, J_{O2} was significantly higher in the wild type than in TL46, which was higher than in TL25 (Fig. 3).

Stimulation of CO_2 Assimilation by 2 kPa O_2

The effect of photorespiration on *A* was determined by reducing the O_2 levels in the leaf chamber from 20 to 2 kPa and assessing the percentage stimulation of CO_2 assimilation by low O_2 [$(A_2/A_{20} - 1) \times 100$]. Under 70 Pa CO_2 and a PPFD of 800 μmol m^{-2} s^{-1}, line TL46 exhibited reversed O_2 sensitivity (inhibition of CO_2 assimilation by 2 kPa O_2),

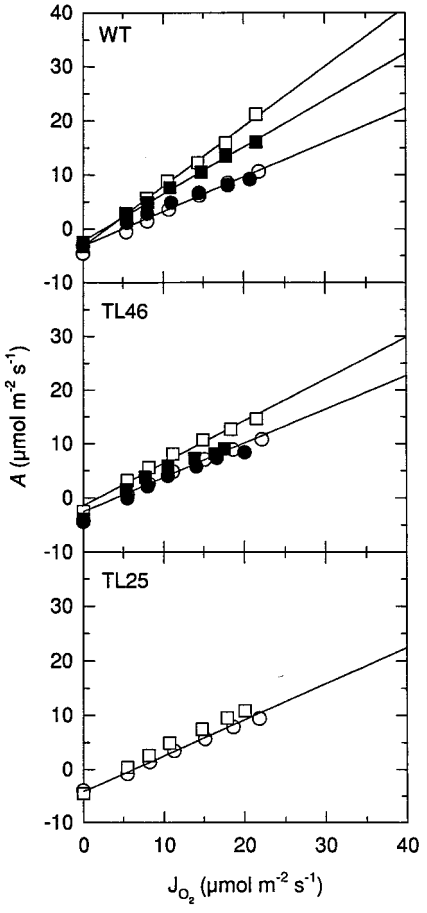

Figure 2. The relationship (initial slope) between J_{O2} and the A in Arabidopsis wild type (WT), TL46, and TL25. The plants were grown under a PPFD of either 100 (●, ■) or 350 (○, □) μmol m^{-2} s^{-1}. CO_2 assimilation was conducted under various PPFD regimes at 25°C, an O_2 partial pressure of 20 kPa, and a CO_2 partial pressure of either 31 Pa (○, ●) or 80 Pa (□, ■). Symbols represent means of measurements of two plants.

whereas the wild type still exhibited partial stimulation of CO_2 assimilation by low O_2 (Fig. 4). Under 35 Pa CO_2 and a PPFD of 800 μmol m^{-2} s^{-1}, line TL46 exhibited less stimulation of CO_2 assimilation than the wild type when O_2 levels were reduced from 20 to 2 kPa (data not shown). The loss of O_2 sensitivity in the mutant is consistent with the loss of CO_2 sensitivity described above (Fig. 1), indicating that the mutants are more feedback inhibited as a result of reduction in capacity for starch synthesis.

$^{14}CO_2$ Partitioning into Starch and Suc

The steady-state levels of in vivo starch synthesis were assessed in a special leaf chamber designed to feed $^{14}CO_2$ to whole Arabidopsis plants while simultaneously monitoring A with an IR CO_2 gas analyzer. After a steady-state rate of CO_2 assimilation was attained, the leaf chamber was gassed with $^{14}CO_2$ for 10 min under a PPFD of 800 μmol m^{-2} s^{-1} at 31 or 80 Pa CO_2, and then chased for 10 min.

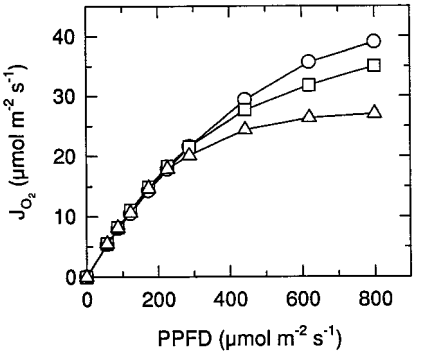

Figure 3. J_{O2} at 80 Pa CO_2 at 25°C and various PPFD regimes in Arabidopsis wild-type (○), TL46 (□), and TL25 (△) plants grown at high light (350 μmol m^{-2} s^{-1}). Symbols represent means of measurements of two plants.

Overall, in wild-type plants the rate of starch and Suc synthesis was higher in high-light-grown than in low-light-grown plants. Similarly, carbohydrate synthesis was higher at 80 Pa CO_2 than at 31 Pa CO_2 (Table II). However, the relative partitioning of $^{14}CO_2$ incorporation into starch and Suc differed at these two CO_2 concentrations. At 80 Pa, the incorporation of $^{14}CO_2$ into starch was about 2-fold higher than $^{14}CO_2$ incorporation into Suc. At the lower CO_2 concentration, there was a large decrease in the ratio of $^{14}CO_2$ incorporation into starch versus Suc in the wild-type plants.

The starch mutants showed lower rates of $^{14}CO_2$ incorporation into starch, especially under saturated light and CO_2 conditions, compared with the wild type (Table II). Under 80 Pa CO_2, starch-labeling rates by high-light-grown TL46 and TL25 were about 40% and 14%, respectively, of the wild-type levels. It is interesting that the reduced incorporation of $^{14}CO_2$ into starch by the mutants was partially compensated for by increased incorporation into Suc. This is clearly evident for the starch-deficient mutant TL46,

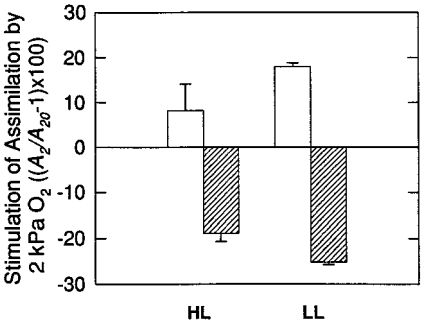

Figure 4. Stimulation of CO_2 assimilation by 2 kPa O_2 in Arabidopsis wild type (open bars) and TL46 (striped bars). CO_2 assimilation was conducted under a PPFD of 800 μmol m^{-2} s^{-1} at 25°C and CO_2 partial pressure of 70 Pa. LL, Plants were grown at low light (100 μmol m^{-2} s^{-1}); HL, plants were grown at high light (350 μmol m^{-2} s^{-1}). Symbols represent means of measurements of two plants. A_2, CO_2 assimilation at 2 k Pa O_2; A_{20}, CO_2 assimilation at 20kPa O_2. Bars indicate SD.

Table II. *Carbon partitioning into starch and Suc after a 10-min pulse and a 10-min chase*

$^{14}CO_2$ feeding was conducted under a PPFD of 800 μmol m^{-2} s^{-1} at a leaf temperature of 25°C. Plants were assayed near initiation of bolting; the average age was 31 and 36 d for plants grown under a PPFD of 350 and 100 μmol m^{-2} s^{-1}, respectively; $n = 2$. WT, Wild type; HL, high light; LL, low light.

Plant	Growth Light	Measuring CO_2	Net CO_2 Uptake (*A*)	Total ^{14}C Fixed	Starch	Suc	Ratio of Starch/Suc
		Pa		*μmol C m^{-2} s^{-1}*			
WT	HL	31	17.4 ± 1.2	18.3 ± 1.1	6.3 ± 1.4	8.6 ± 0.6	0.73
TL46	HL	31	15.3 ± 0.6	16.6 ± 1.0	5.2 ± 0.3	7.8 ± 0.5	0.67
TL25	HL	31	14.4 ± 0.3	N.D.[a]	1.6 ± 0.5	10.2 ± 0.1	0.16
WT	HL	80	33.4 ± 0.9	31.3 ± 1.7	18.6 ± 2.6	9.7 ± 0.7	1.92
TL46	HL	80	23.9 ± 1.0	24.9 ± 2.7	7.5 ± 2.0	13.8 ± 0.6	0.54
TL25	HL	80	15.8 ± 1.4	N.D.	2.6 ± 0.9	11.0 ± 0.1	0.24
WT	LL	31	10.6 ± 0.3	11.8 ± 0.7	4.8 ± 0.3	3.2 ± 0.4	1.50
TL46	LL	31	9.6 ± 0.1	10.5 ± 0.5	3.0 ± 0.2	3.8 ± 0.2	0.79
WT	LL	80	23.9 ± 4.2	22.4 ± 2.5	11.8 ± 1.0	6.6 ± 0.9	1.79
TL46	LL	80	11.4 ± 2.1	13.1 ± 0.1	3.6 ± 0.8	7.0 ± 0.5	0.51

[a] N.D., Not determined.

but less so for the near-starchless mutant TL25. Low-light-grown wild-type and TL46 plants showed similar patterns of partitioning of $^{14}CO_2$ into Suc and starch, although absolute incorporation levels were reduced, particularly for Suc. Under 31 Pa CO_2, there was a 10% to 20% reduction in net CO_2 uptake in the mutants compared with the wild type. Again, there was evidence for the reduction in partitioning into starch being partially compensated for by increased partitioning into Suc. The $^{14}CO_2$ partitioning patterns into starch and Suc were similar between plants pulsed for 10 min and then chased for 1 or 10 min (data not shown).

There were significant correlations between *A* and total $^{14}CO_2$ incorporation into leaves (Fig. 5A) and between *A* and the rate of starch synthesis (Fig. 5B). In contrast, there was no correlation between *A* and the rate of Suc synthesis ($P > 0.05$) (Fig. 5C). Starch synthesis increased more rapidly with increasing *A* (slope = 0.6) than Suc synthesis (slope = 0.3). These results suggested that starch synthesis would become very low if *A* was less than 7 μmol m^{-2} s^{-1}, which agrees with a previous report with bean (Sharkey et al., 1985).

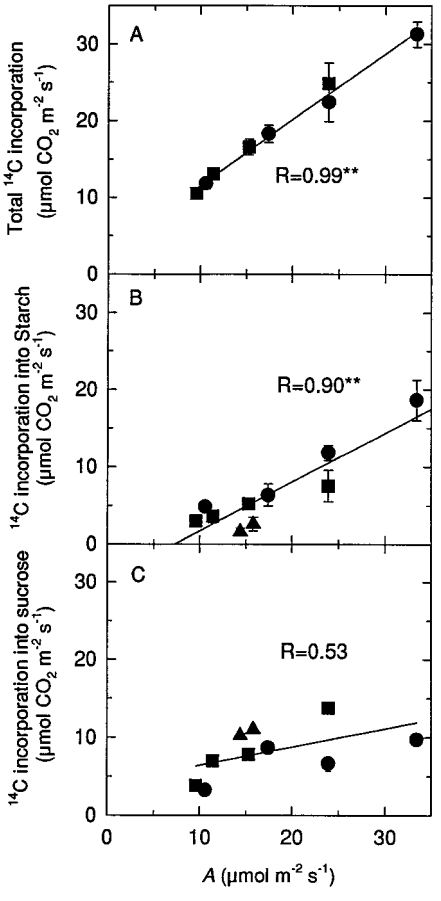

Figure 5. The relationships between CO_2 assimilation rate, total ^{14}C incorporation, starch, and Suc synthesis in Arabidopsis. CO_2 assimilation was measured under a PPFD of 800 μmol m^{-2} s^{-1} at 25°C and various CO_2 partial pressures in wild type (●), TL46 (■), and TL25 (▲). Different points for each genotype are the results of different CO_2 and light levels (see Table II). Asterisks indicate $P < 0.01$. Bars indicate SD.

Cumulative Leaf Area

Leaf-area development as an indicator of growth was examined under the two PPFD growth regimes. Under a 12-h light/12-h dark photoperiod, both wild-type and TL46 plants exhibited similar increases in leaf area during the growth period (Fig. 6). In contrast, the near-starchless mutant, TL25, grew much more slowly. Also, under high light TL25 took 10 d more to initiate flowering compared with the wild type and TL46.

There was a significant correlation between the rate of starch synthesis and the cumulative leaf area per plant (Fig. 7). These results suggest that there is a minimum requirement for starch synthesis for normal plant growth and development.

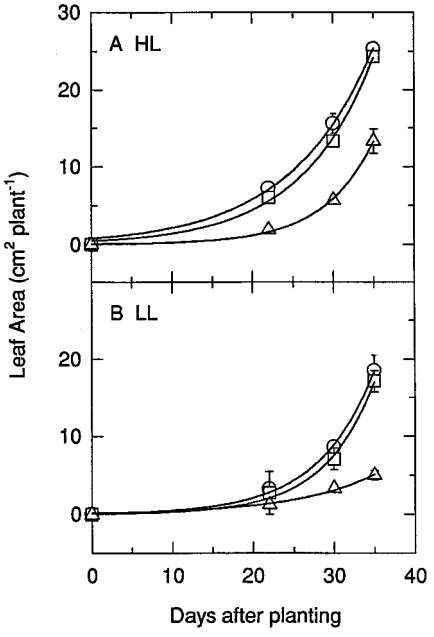

Figure 6. Cumulative leaf area during growth of Arabidopsis wild-type (○), TL46 (□), and TL25 (△) plants. The plants were grown under a PPFD of either 100 (A) or 350 (B) μmol m^{-2} s^{-1} and a photoperiod of 12/12 h (light and dark, respectively). Symbols represent means of measurements of three plants. Bars indicate SD.

DISCUSSION

Deficiencies in Starch Synthesis Result in Feedback of Photosynthesis

The results presented in this study demonstrate clearly that leaf starch metabolism has a very significant effect on the photosynthetic capacity of the plant. Wild-type Arabidopsis plants have higher *A* than the starch mutants TL46 and TL25 under high CO_2 over a wide range of PPFD conditions (Fig. 1). Although these differences in *A* were more readily evident in high-light-grown plants under saturating CO_2, a similar trend was also observed for low-light-grown plants.

The rates of J_{O2} and *A* were strongly correlated (Fig. 2). At a given J_{O2}, enhancement of *A* at elevated levels of CO_2 attributable to inhibition of photorespiration was higher in the wild type than in TL46, which in turn was higher than in TL25. At atmospheric levels of CO_2 and O_2, photorespiration contributes significantly to Pi recycling in C_3 plants (Harley and Sharkey, 1991; Eichelmann and Laisk, 1994). When CO_2 levels increase, photorespiration, which acts as an electron acceptor, declines and starch synthesis becomes important for Pi recycling, as indicated by the large increase in starch synthesis under high CO_2. The wild type had a higher capacity for starch synthesis and, thus, retained higher Pi recycling, electron transport, and higher CO_2 assimilation than the starch mutants.

At a PPFD above 300 μmol m^{-2} s^{-1}, J_{O2} was significantly higher in the wild type than in TL46, which in turn was higher than in TL25 (Fig. 3). These results indicate that there is feedback regulation of electron transport by deficiencies in starch synthesis. Similar observations were described for the tobacco plastid phosphoglucomutase mutant, which is defective in starch synthesis and has a lower rate of electron transport (Peterson and Hanson, 1991; Eichelmann and Laisk, 1994).

O_2 Sensitivity Is a Good Indicator of Feedback Inhibition

CO_2 assimilation is often stimulated by subatmospheric levels of O_2 in C_3 plants because of the reduced oxygenase activity of the bifunctional enzyme Rubisco (Sage and Sharkey, 1987). However, in some cases, such as at low temperatures (Leegood and Furbank, 1986; Sage and Sharkey, 1987; Sun et al., 1997) or at high CO_2 levels (Viil et al., 1977; Sharkey, 1985), C_3 plants exhibit O_2-insensitive photosynthesis or even reversed O_2 sensitivity. Reversed O_2 sensitivity is also seen in a *Flaveria* mutant that contains reduced levels of the cytosolic Fru bisphosphatase (Sharkey et al., 1995) and in a tobacco starchless mutant with phosphoglucomutase deficiency (Hanson, 1990; Eichelmann and Laisk, 1994). The occurrence of O_2-insensitive photosynthesis has been suggested to be caused by limitations in triose-P utilization, which causes a Pi limitation and its concomitant effects on reducing ATP and ribulose-1,5-bisphosphate regeneration (Sharkey et al., 1995). Reversed O_2 sensitivity (i.e. inhibition of CO_2 fixation by low O_2) is poorly understood, but according to one hypothesis it is associated with the loss of a photorespiratory Pi-generating mechanism in photosynthesis under low O_2, when the capacity for utilization of triose-P is limiting (Harley and Sharkey, 1991). Perhaps the limitation on conversion of triose-P to starch biosynthesis in the Arabidopsis mutants and the associated release of Pi could result in its having a greater Pi deficiency when photorespiration is eliminated under low O_2. It has also been suggested that reversed O_2 sensitivity may occur by accumulation of 3-PGA and its inhibition of starch synthesis by inhibition of phosphoglucoisomerase (Sharkey and Vassey, 1989); however, this would not account for the mutant, which cannot make starch, being more susceptible to reversed sensitivity than the wild type. Eichelmann

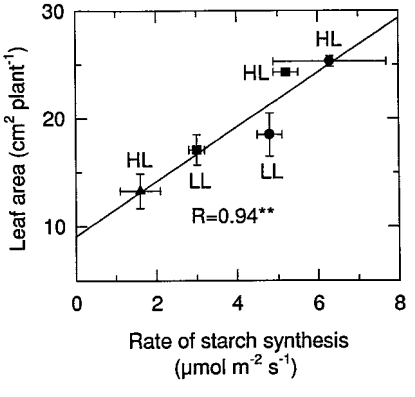

Figure 7. The relationship between the rate of starch synthesis and total leaf area per plant 31 to 36 d after planting in Arabidopsis wild type (●), TL46 (■), and TL25 (▲). LL, Plants were grown at low light (100 μmol m^{-2} s^{-1}); HL, plants were grown at high light (350 μmol m^{-2} s^{-1}). Data for starch synthesis are from Table II. Asterisks indicate $P < 0.01$; bars indicate SD.

and Laisk (1994) suggested that the reversal of O_2 sensitivity in a tobacco mutant impaired in starch synthesis is caused by Pi depletion, but they were unable to explain this effect with the hypotheses described above.

Our results support the view that the loss of O_2 sensitivity observed in starch synthesis mutants denotes feedback limitation attributable to decreased potential to utilize additional triose-P for carbohydrate synthesis when shifting from 20 to 2 kPa O_2. Under atmospheric CO_2 levels, TL46 exhibited less stimulation of CO_2 assimilation at 2 kPa O_2 than did the wild type. Under 70 Pa CO_2, TL46 exhibited reversed O_2 sensitivity (inhibition of CO_2 assimilation by reduction from 20 to 2 kPa O_2), whereas the wild type showed partial stimulation of CO_2 assimilation by low O_2 (Fig. 4). This inability to utilize additional triose-P for carbohydrate synthesis causes Pi cycling to become limited for ATP and ribulose-1,5-bisphosphate regeneration, which reduces both electron transport and CO_2-assimilation rates (Figs. 1–3).

Partitioning of $^{14}CO_2$ Assimilation between Suc and Starch

Rates of $^{14}CO_2$ incorporation into Suc and starch were dependent on CO_2 levels and the previous growth conditions of the plants. At atmospheric CO_2 (31 Pa), almost one-half of the total $^{14}CO_2$ incorporated was partitioned into Suc, with a smaller proportion (34% of the total) allocated into starch. Although the total $^{14}CO_2$-incorporation rate was lower in TL46 than in the wild type, TL46 showed a similar distribution between Suc and starch as the wild type. In TL25 the bulk of the $^{14}CO_2$ was incorporated into Suc with very little labeling into starch, a pattern consistent with its starchless phenotype.

At higher CO_2 levels (80 Pa), total $^{14}CO_2$-incorporation rates were increased for the wild type and TL46. In the wild type most of this increase in $^{14}CO_2$ incorporation was partitioned into starch, with little increase in Suc. These results indicate that Suc biosynthesis is saturated or close to being saturated at atmospheric CO_2 and high light levels in the wild type (see also Fig. 5). Conversely, a different pattern was evident for TL46 under these same conditions. The bulk of the limited increase in $^{14}CO_2$ incorporation was partitioned into Suc, with smaller amounts into starch. Hence, with the restricted capacity of TL46 to synthesize starch, there was increased shunting of triose-P into Suc under high CO_2, although the rate of photosynthesis was still limited by triose-P utilization.

TL46 showed a $^{14}CO_2$ incorporation rate into starch that was 60% to 80% of that in the wild type at atmospheric levels of CO_2, or 30% to 40% of that in the wild type at 80 Pa CO_2 (Table II). The $^{14}CO_2$ incorporation rates were consistent with the levels of starch (40%–50% of the wild-type levels) reported for TL46 when grown at atmospheric levels of CO_2 and a PPFD either at 120 μmol m^{-2} s^{-1} (Lin et al., 1988a) or 600 μmol m^{-2} s^{-1} (Schulze et al., 1991). The $^{14}CO_2$ incorporation rates reported here and the steady-state starch levels reported elsewhere (Lin et al., 1988a; Schulze et al., 1991), however, are not consistent with the low activity of AGPase (7% of that in the wild type) reported for TL46 (Lin et al., 1988a; Neuhaus and Stitt, 1990). Results from recent biochemical and genetic studies on the structure function of AGPase can account for this apparent discrepancy. AGPase is composed of two subunit types, a large subunit (regulatory) and a small subunit (catalytic) (Okita et al., 1990; Ballicora et al., 1995). The large subunit is unable to form a functional enzyme by itself, whereas the small subunit is capable of forming an active enzyme even in the absence of the large subunit, albeit with reduced sensitivity to allosteric regulation. TL46 contains a missense mutation in the large subunit (Wang et al., 1997), resulting in the formation of an active small subunit enzyme that requires much higher levels of 3-phosphoglyceric acid for maximum enzyme activity and is much more sensitive to inhibition by Pi than the wild-type enzyme (Li and Preiss, 1992). Because limiting amounts of 3-PGA (1–2 mM) were used to measure AGPase activities (Lin et al., 1988a; Neuhaus and Stitt, 1990), the activities are likely underestimated.

The relative $^{14}CO_2$ incorporation rates into starch and Suc we determined for TL46 differ substantially from those reported earlier by Neuhaus and Stitt (1990). In their study, under high CO_2 TL46 displayed only 9% of the wild-type rate of $^{14}CO_2$ incorporation into starch, compared with the 30% rate reported here. Moreover, whereas an increase in $^{14}CO_2$ incorporation into Suc was observed at higher CO_2 levels in TL46 compared to the wild type (Table II), they reported a reduction in Suc synthesis. Several factors may account for the discrepancy between these results. First, Neuhaus and Stitt (1990) used a detached leaf-disc assay of photosynthesis with an O_2 electrode under very high CO_2, whereas we used a nondestructive whole-plant assay. When leaves are cut, Suc accumulation and wounding may occur, impairing photosynthesis and carbon partitioning. Therefore, Suc transport out of the leaves is prevented, as in girdling of the leaf petiole by hot wax (Goldschmidt and Huber, 1992) or cold girdling (Krapp and Stitt, 1995). Evidence in support of this view is that A during $^{14}CO_2$ labeling was about two to three times higher in our study than in that of Neuhaus and Stitt (1990), and we have observed lower CO_2-saturated rates of photosynthesis in Arabidopsis using the leaf-disc system than with intact plants (M. Poulson and G. Edwards, unpublished data). A second major factor is that a mature leaf (used in the leaf-disc assay) versus the whole plant (used in the present study) can differ in carbon partitioning. For example, in cassava the Suc content is very similar in leaves of different ages, whereas starch levels are much higher (up to severalfold) in young leaves than in old leaves (Angelov et al., 1993). This results in the whole plant having a higher starch-to-Suc ratio than the mature leaves alone. Third, because long photoperiods can reduce partitioning into starch (Chatterton and Silvius, 1979), plants grown under the longer photoperiods (16/8 h) used by Neuhaus and Stitt (1990) may have lower starch synthesis than the plants in the current study (12/12 h).

Growth light also affected partitioning into Suc and starch. High-light-grown wild-type plants had higher AGPase activity and high rates of CO_2 fixation, with a larger increase in partitioning into starch under high CO_2 (80 Pa), than the low-light-grown plants. However, high-light-grown TL46 had an AGPase activity similar to that of

low-light-grown plants, and the higher rate of photosynthesis in high-light-grown plants under high CO_2 was largely accounted for by increased partitioning into Suc.

Starch Synthesis Is Regulated and Related to Photosynthetic Capacity

Eichelmann and Laisk (1994) and Stitt (1996) have suggested that starch is an "overflow" product when photosynthesis is high. Based on calculations from a biochemical model, Eichelmann and Laisk (1994) showed that when photosynthesis increases, Suc synthesis saturates first and then the capacity of starch synthesis is triggered. There is also evidence for this in C_4 plants, since the ratio of $^{14}CO_2$ incorporation into starch/Suc increases with increasing PPFD (Lunn and Hatch, 1997). Other studies have indicated that starch synthesis is "programmed." For example, a transient increase (3–4 d) in starch level in the leaves when shifting to shorter days has been seen as a programmed response meeting the increased need for carbon during the lengthened night (Geiger et al., 1985). The ability of leaves to synthesize starch when photosynthetic rates are low, such as under low light and low CO_2 (Silvius et al., 1979; Lin et al., 1988b; Schulze et al., 1991; Huber and Hanson, 1992), further supports the programmed model for starch synthesis.

The programmed and the overflow models are not mutually exclusive, and both mechanisms may operate depending on the environmental conditions. The overflow model is operative in Arabidopsis, as indicated by the results from the $^{14}CO_2$ labeling study. When CO_2 levels were elevated from 31 to 80 Pa, Suc synthesis increased only marginally (18%), whereas starch synthesis increased by almost 3-fold in the wild type (Table II). Hence, increased triose-P production by increased CO_2 assimilation at 80 Pa CO_2 is mainly partitioned into starch, a process that is facilitated by the allosteric activation of AGPase by increased 3-PGA and decreased Pi levels (Preiss, 1982).

The programmed model is likely constitutive and important for plant growth during a day/night diurnal regime. Cytosolic Fru-1,6-bisphosphatase regulation through Fru-2,6-bisP (Stitt, 1996) and Suc-P synthase phosphorylation (Huber and Huber, 1992) likely play important roles in programmed starch synthesis. To some extent the programmed model may apply to the present results with Arabidopsis, because there was significant starch synthesis at atmospheric levels of CO_2 (Table II), a level that was limiting for photosynthesis (Fig. 1).

There is a strong correlation between rates of starch synthesis and CO_2 assimilation when measurements are made under varying CO_2 levels with plants grown at different light levels and having different AGPase activities (Fig. 5). These observations indicate the importance of starch synthesis in photosynthesis. In the wild type, the rate of starch synthesis was far from saturation at atmospheric levels of CO_2, and increased by about 3-fold when shifting to 80 Pa CO_2, whereas in TL25 and TL46 plants starch synthesis was near saturation at the lower level of CO_2 (Table II). In addition, reduction in starch synthesis in AGPase mutants was only compensated for to a small degree by increased Suc synthesis (Table II). Thus, the wild type had a higher capacity to utilize triose-P to recycle Pi and prevent feedback inhibition and, thus, to accommodate the potential for increased CO_2 fixation in the C_3 cycle.

Growth under a Day/Night Diurnal Regime Is Starch Dependent

Cumulative leaf area as a measure of growth under a 12-h photoperiod was significantly reduced in line TL25, whereas there was only an apparent slight reduction in TL46, compared with the wild type (Fig. 6). CO_2 assimilation rates under atmospheric levels of CO_2 were only slightly lower in line TL25 than in the wild-type or TL46 plants (Table II). Thus, lower starch synthesis in TL25 (Table II) can be considered to account for its slower growth. Starch is an important carbon source for growth at night, when most leaf expansion occurs (see Huber and Hanson, 1992). Typically, the starch level in the leaves increases linearly with time during the day and decreases linearly during the night, and starch synthesized during the day equals starch degraded during the night (Lin et al., 1988a, 1988b). At 800 μmol m^{-2} s^{-1} PPFD, TL46 had a rate of starch synthesis 60% to 80% of the wild type at atmospheric levels of CO_2 and a rate of starch synthesis 30% to 40% of the wild type at 80 Pa CO_2. Because TL46 grown under atmospheric levels of CO_2 at 100 or 350 μmol m^{-2} s^{-1} PPFD had a cumulative leaf area only slightly lower than that of the wild type, this suggests that it produced sufficient starch under those conditions.

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