Photosynthesis and Carbon Partitioning in Transgenic Tobacco Plants Deficient in Leaf Cytosolic Pyruvate Kinase

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Whole-plant diurnal C exchange analysis provided a noninvasive estimation of daily net C gain in transgenic tobacco (Nicotiana tabacum L.) plants deficient in leaf cytosolic pyruvate kinase (PKc,PKc−). PKc− plants cultivated under a low light intensity (100 μmol m−2 s−1) were previously shown to exhibit markedly reduced root growth, as well as delayed shoot and flower development when compared with plants having wild-type levels of PKc (PKc+,PKc− and PKc+, source leaves showed a similar net C gain, photosynthesis over a range of light intensities, and a capacity to export newly fixed 14CO2 during photosynthesis. However, during growth under low light the nighttime, export of previously fixed 14CO2 by fully expanded PKc− leaves was 40% lower, whereas concurrent respiratory 14CO2 evolution was 40% higher than that of PKc+, leaves. This provides a rationale for the reduced root growth of the PKc− plants grown at low irradiance. Leaf photosynthetic and export characteristics in PKc− and PKc+, plants raised in a greenhouse during winter months resembled those of plants grown in chambers at low irradiance. The data suggest that PKc, in source leaves has a critical role in regulating nighttime respiration particularly when the available pool of photoassimilates for export and leaf respiratory processes are low.

PK catalyzes the synthesis of pyruvate and ATP from PEP and ADP and is believed to be a major control point of plant and nonplant glycolysis (Plaxton, 1996). The enzyme has been demonstrated to be significantly displaced from equilibrium in vivo and has pronounced regulatory properties in vitro. Plant PK exists as cytosolic and plastidic isozymes (PKc and PKp, respectively), which differ substantially in their molecular and kinetic/regulatory properties. PKp plays an important role in generating the precursor pyruvate for various biosynthetic pathways and mitochondrial respiration. The biosynthetic role of cytosolic glycolysis is central in actively growing autotrophic tissue (Plaxton, 1996), in which a significant proportion of the C that enters the glycolytic pathway is incorporated into numerous compounds such as amino acids, fatty acids, and secondary metabolites. The exact contribution that these enzymatic steps provide in source leaves and in developing sink tissues remains unclear.

PK deficiency in nonplant species causes serious detrimental effects. However, our earlier studies revealed that transgenic tobacco plants (Nicotiana tabacum L.) deficient in leaf PKc (PKc−) grew from seed to seed, demonstrating the remarkable flexibility of plant PEP metabolism (Gottlob-McHugh et al., 1992; Knowles et al., 1998). Plant cells can use a variety of alternative metabolic routes to directly or indirectly circumvent the reaction catalyzed by PKc. These could include the action of PEP phosphatase or the combined action of PEP carboxylase, malate dehydrogenase, and NAD-malic enzyme (Plaxton, 1996). It is also possible that the elevated levels of PEP observed in the PKc− leaves (Gottlob-McHugh et al., 1992) results in an increased flux of glycolytic C from the cytosol to the chloroplast where the PEP may be metabolized by PKp. Elimination of leaf PKc can alter C metabolism and growth when total C supply is limited by growing the plants at reduced PPFD. Knowles et al. (1998) showed that, when grown at low PPFD, the PKc− tobacco exhibited a delayed shoot and flower development, as well as a striking reduction in root growth. Since the lack of PKc and the resulting altered glycolytic activity appeared to be confined to the leaves, we decided to further investigate the role of the leaves as sources of reduced C.

We recently described a procedure for evaluating immediate export rates during photosynthesis so that we could test and differentiate between the effect of environmental challenges, such as leaf warming, on the ability of the sources leaves to fix CO2 and to export the reduced C products (Jiao and Grodzinski, 1996; Leonardos et al., 1996). These protocols were modified to study the effect of short- and long-term CO2 enrichment on photosynthesis and export rates in source leaves of a number of C3 and C4 species (Jiao and Grodzinski, 1996; 1998; Grodzinski et al., 1998). The aim of the present study was to use these procedures to assess whole-plant gas exchange, photosynthesis, respiration, and export in intact, attached source leaves of two independent homozygous PKc− tobacco lines. We

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Abbreviations: GM, Geiger-Müller; NCER, net carbon exchange rate; PK, pyruvate kinase; PKc, PKp, cytosolic PK and plastid PK, respectively; PKc− and PKp+, tobacco plants that are and are not deficient in leaf PKc, respectively.

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report that, when both PKc− lines were cultivated under low irradiance, nighttime export of recently fixed CO2 was reduced, whereas concurrent respiration of 14C assimilates was enhanced. These findings provide a rationale for the reduced root development of the PKc− plants.

**MATERIALS AND METHODS**

**Plant Materials**

Two transgenic tobacco (*Nicotiana tabacum* L.) lines that specifically lacked PKc in their leaves were obtained because of the trans-inactivation phenomenon known as cosuppression (Gottlob-McHugh et al., 1992). Selfing of each parent line resulted in two PKc− progeny lines, 14-1 and 15-7, in which the cosuppression was relatively stable, and a PKc+ line, 18-7, which contained wild-type levels of PKc (Knowles et al., 1998). The seeds obtained from the selfing of 14-1 and 15-7 (designated PKc−1 and PKc−2, respectively) plus the wild type and 18-7 (PKc+1 and PKc+2, respectively) were used in the present study. Seeds were germinated and grown in PROMIX-BX (Les Toubières Premier LTÉE, Rivière du Loup, QC, Canada) in 20-cm pots in growth chambers at the University of Guelph. A 16-h photoperiod was maintained at 22°C ± 1°C during which the PPFD (400–700 nm) was 500 μmol m−2 s−1 (moderate light) or 100 μmol m−2 s−1 (low light). During the 8-h dark period the temperature was 18°C ± 1°C. Plants were fertilized biweekly with a nutrient solution, as described previously (Knowles et al., 1998).

In other experiments plants were grown during winter months (i.e. between November and March) in a greenhouse at the University of Guelph (latitude approximately 43.5°N). Solar-generated PPFD varied from 50 μmol m−2 s−1 on overcast days to more than 1000 μmol m−2 s−1 on sunny days. Artificial lighting was supplied by 1000-W Sylvania metal halide lamps throughout the 16-h photoperiod and maintained a minimum daytime PPFD of about 130 μmol m−2 s−1 at the plant level. Temperatures in the experimental greenhouse compartments were typically 24°C ± 1°C/18°C ± 1°C, day (16 h)/night (8 h).

**PK Assay and Immunoblot Analyses**

All measurements were made on the most recently fully expanded leaves, which were harvested, frozen in liquid N2, and stored at −80°C until used. Enzyme extracts were prepared from leaves, as described previously (Gottlob-McHugh et al., 1992). PK activity was assayed spectrophotometrically at 25°C, as described by Plaxton (1989), and was corrected for PEP phosphatase activity by omitting ADP from the reaction mixture. One unit of PK activity is defined as the amount of enzyme resulting in the utilization of 1 μmol PEP min−1. Activity values represent the means of quadruplicate determinations conducted with three separate extracts and were reproducible to within ±10% se. Protein concentration was determined by the modified Bradford assay (Bollag and Edelstein, 1991) using bovine γ-globulin as the standard. Extracts were electrophoresed on 7.5% (w/v) SDS-polyacrylamide minigels and electroblotted onto a PVDF membrane. Immunoblotting was performed using affinity-purified anti-castor endosperm PKc−IgG, and antigenic polypeptides were detected using an alkaline-phosphatase-conjugated secondary antibody, as described previously (Plaxton, 1989). Immunological specificities were confirmed by conducting immunoblots in which rabbit preimmune serum was substituted for the anti-PKc−IgG.

**Whole-Plant NCER and Daily C Gain**

The growth rate of the plants was measured noninvasively by determining the whole-plant NCER using whole-plant gas-exchange chambers, as described previously (Dutton et al., 1988). Measurements were made at 24°C ± 1°C/18°C ± 1°C, day (16 h)/night (8 h), and ambient CO2 (35 Pa) and O2 (21 KPa). Plants grown under the moderate lighting regime were measured at 500 μmol m−2 s−1 PPFD at the level of the top leaf, whereas the plants grown under low light were measured at 100 μmol m−2 s−1 PPFD. One plant per chamber was used for the measurements. Because four chambers were run concurrently using a central IR gas-analysis system, CO2 exchange measurements were made at 6- to 8-min intervals during a typical 50-h test (Leonardos et al., 1994). Six replications were conducted and based on the average diurnal gas exchange measurements; whole-plant daily C gain was calculated for each line under both PPFD levels.

**Leaf Net CO2 Exchange Rates**

Plants were illuminated with Sylvania metal halide lamps (1000 W), which could provide a maximum of about 1800 μmol m−2 s−1 PPFD at the leaf level. The light response curves for photosynthesis were derived by using a series of neutral screens to reduce PPFD. The leaf gas-exchange rates of the most recently expanded leaves of 8- to 9-week-old plants were measured using an open-flow system described previously (Jiao and Grodzinski, 1996). Both leaf and air temperature in the plant chamber were maintained at 24°C ± 1°C. The inlet gas contained 35 Pa CO2 and 21 KPa O2. Chlorophyll content was determined as described by Wintermans and de Mot (1965).

**Export and Storage of 14C Assimilates during Photosynthesis**

Export of newly fixed 14C assimilates during steady-state photosynthesis was estimated as described previously (Jiao and Grodzinski, 1996). Plants were illuminated with Sylvania metal halide lamps (1000 W), which provided about 1200 μmol m−2 s−1 PPFD at the leaf level. A GM detector was mounted in the lower half of the leaf cuvette to continuously monitor radioactivity (i.e. 14C accumulation) in the source leaves. To establish the time required to reach an equilibrium between the 14CO2 of known specific activity and the 14C-labeled Suc pool, leaves were first fed with 14CO2 from 30 to 120 min and the labeled products were analyzed. The fed leaf was extracted with boiling 80% ethanol:water (v/v), and the major 14C-labeled assimilates...
were analyzed as described elsewhere (Jiao and Grodzinski, 1996). During feeding periods net 14CO2 assimilation and 14C accumulation rates of attached intact leaves were monitored continuously in a noninvasive manner using an IR gas and a GM detector, respectively. The measurement of the export rate of the newly fixed 14CO2 during steady-state photosynthesis was calculated as the difference between the rate of 14CO2 assimilation and the retention of 14C assimilates 90 to 120 min after 14CO2 feeding began. As reported below, isotopic equilibrium between the specific activity of the 14C-Suc in the leaf and that of the 14CO2 in the gas stream was established during this period. The CO2 and O2 levels were 35 Pa and 21 KPa, respectively. Whole-plant and leaf temperatures were 24°C ± 1°C and the RH was approximately 70%.

Leaf Dark Respiration and Nighttime Export of 14C-Labeled Assimilates

The respiration and export of 14C assimilates during the dark period that followed the 14CO2 feeding were determined by trapping the 14CO2 released and continuing to monitor the level of 14C in the source leaf with the GM detectors mounted in the leaf cuvettes (Jiao and Grodzinski, 1998). After the 14CO2 was supplied for 2 h and the rates of photosynthesis and concurrent export were determined, the lamps were extinguished and the leaves were supplied with a gas stream lacking 14CO2. The CO2 and O2 levels were 35 Pa and 21 KPa, respectively. Whole-plant and leaf temperatures were 24°C ± 1°C and the RH was approximately 70%.

RESULTS

PK Activity and Immunoblot Analysis

PK activity assays and immunoblotting using anti-castor endosperm PKc-IgG were used to assess the relative abundance of PKc in extracts prepared from leaves of the same tobacco plants used in the physiological studies described below. The PK activities of fully expanded PKc+ and PKc− leaves harvested from plants grown under moderate or low light are reported in Figure 1. Extractable PK activity of the PKc− leaves was reduced by 70% to 85% relative to the PKc+ controls. Two to three separate extracts of the PKc+ and PKc− leaves were analyzed by immunoblotting, and representative results are shown in Figure 1. Immunoblots of PKc+ leaf extracts revealed an intense immunoreactive polypeptide at 57 kD, which corresponds to subunits of tobacco leaf PK, (Knowles et al., 1998). By contrast, antigenic staining of PKc on immunoblots of PKc− leaf extracts was either very faint (PKc−) or undetectable.

Plant Growth, Leaf Area, and Chlorophyll Content

As reported previously (Knowles et al., 1998), the PKc− lines had a slower rate of development than did the two PKc+ lines. Therefore, plants that were at an identical developmental stage were compared. The data reported here are from the stage at which the first flower bud was visible. Total leaf area per plant of the four lines grown under 500 μmol m−2 s−1 PPFD (moderate light) were similar (Table I). However, when plants were raised under 100 μmol m−2 s−1 PPFD (low light), the total leaf area of the PKc− was less than that of the PKc+ controls. When leaf chlorophyll content of the PKc− and PKc+ lines were compared at a single light condition, the leaves of the PKc− lines had more chlorophyll than did the leaves of the PKc+ plants (Table I). Under moderate light leaves of each line had a greater chlorophyll content than leaves of that same line had when grown under low light (Table I).

Whole-Plant NCER and Daily C Gain

Figure 2 shows whole-plant NCER and daily net C gain of each tobacco line grown and measured at 500 or 100 μmol m−2 s−1 PPFD. Within each panel it is clear that photosynthesis at moderate light was greater than that at low light. However, whole-plant photosynthesis of the four lines was similar when compared at the same PPFD level (Fig. 2, A–D). The photosynthesis rates for PKc−1 and PKc−2 plants grown and measured at the moderate light intensity were 5.8 and 6.1 μmol C fixed m−2 s−1, respectively. Whole-plant dark respiration rates ranged from 1.2 μmol C released m−2 s−1 in PKc+1 to 1.6 μmol C released m−2 s−1 in PKc−2. At the lower PPFD level, plant photosynthesis rates were one-third that of those at the moderate PPFD level, averaging 1.9 and 2.1 μmol C fixed m−2 s−1 for the PKc−2 (Fig. 2D) and PKc−1 lines, respectively (Fig. 2C). Dark respiration rates of plants grown under low light were also less than those of plants grown under moderate light. For example, in plants acclimated to 100 μmol m−2 s−1 PPFD, the average rates of dark respiration during the 8-h night period were approximately 0.56 and 0.66 μmol C released m−2 s−1 for the PKc+1 (Fig. 2A) and PKc−2 lines (Fig. 2D), respectively.

At 500 μmol m−2 s−1 PPFD the daily C gain of all four tobacco lines was approximately 300 μmol C gained m−2,
which was approximately 3-fold that measured at 100 μmol m$^{-2}$ s$^{-1}$ PPFD (Fig. 2, E–H). The data confirm that at the whole-plant level there was little difference in daily C gain per leaf area among the four tobacco lines.

**Leaf Photosynthesis at Varying Irradiances**

When intact attached source leaves were tested individually, net CO$_2$ fixation rates of PK$_{c}^{2}$ plants were similar to those of the PK$_{c}^{1}$ plants grown at the same light level (Fig. 3). All plants grown at the moderate PPFD exhibited higher rates of leaf photosynthesis (Fig. 3A) than did plants grown at low PPFD (Fig. 3B). The PPFD required to saturate leaf photosynthesis was greater in plants acclimatized to the higher PPFD. In all of the plants grown under moderate and low light, the PPFD required to saturate photosynthesis was approximately 900 and 700 μmol m$^{-2}$ s$^{-1}$, respectively (Fig. 3).

**Establishing Conditions for Estimating Export Rates during Photosynthesis**

The $^{14}$C-labeling patterns in intact attached source leaves from PK$_{-}$ and PK$_{+}$ plants are compared in Figure 4. Leaves of each line that had been grown at low or moderate PPFD were tested at similar stages of plant development at a PPFD that saturates photosynthesis. For ease of reporting the only results shown here are for leaves of the PK$_{+}$ line (Fig. 4, A, C, and E) and the PK$_{-}$ line (Fig. 4, B, D, and F). During the 120-min feed period total $^{14}$CO$_2$ fixation increased linearly (Fig. 4, A and B). The net photosynthetic rates were lower in plants grown at 100 μmol m$^{-2}$ s$^{-1}$ PPFD (refer to Figs. 3 and 5), but net CO$_2$ fixation rates were also constant during the 2-h feed. As illustrated in Figure 4, A and B, the rates of total $^{14}$C retention in the source leaves were higher during the 1st h of the feed and reflects the fact that the label was being randomized among

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**Table I. Total leaf area and chlorophyll content at flowering stage of tobacco plants cultivated under moderate or low light in growth chambers**

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>Leaf Area</th>
<th>Chlorophyll</th>
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<tbody>
<tr>
<td></td>
<td>Moderate light</td>
<td>Low light</td>
</tr>
<tr>
<td></td>
<td>cm$^2$</td>
<td>µg cm$^{-2}$</td>
</tr>
<tr>
<td>PK$_{c}^{+}$</td>
<td>2343 ± 101$^a$</td>
<td>2178 ± 88$^a$</td>
</tr>
<tr>
<td>PK$_{c}^{+}$</td>
<td>2252 ± 131$^a$</td>
<td>2175 ± 96$^a$</td>
</tr>
<tr>
<td>PK$_{-}$</td>
<td>2213 ± 86$^a$</td>
<td>1829 ± 62$^b$</td>
</tr>
<tr>
<td>PK$_{-}$</td>
<td>2208 ± 94$^a$</td>
<td>2083 ± 45$^a$</td>
</tr>
</tbody>
</table>

Each value represents the mean ± se obtained with at least six leaves on six different plants. Significant differences among PK$_{c}^{+}$ and PK$_{-}$ lines by Student’s t test ($P < 0.05$) are indicated by superscript letters (a, b, c).

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**Figure 2.** Whole-plant NCERs and net C gain during a 16-h photoperiod and an 8-h dark period in tobacco: PK$_{c}^{+}$ (A and E); PK$_{c}^{+}$ (B and F); PK$_{-}$ (C and G); and PK$_{-}$ (D and H). NCER was measured when the first flower bud appeared. Plants were both grown and measured at a PPFD of 500 (solid lines) or 100 (dashed lines) μmol m$^{-2}$ s$^{-1}$. The CO$_2$ and O$_2$ levels were 35 Pa and 21 KPa, respectively. Day and night temperatures during the analysis were 22°C and 18°C, respectively. The data are the means of at least four replications.
different intermediate pools before the export pools reached isotopic equilibrium with $^{14}$CO$_2$ in the gas stream.

A major labeled product accumulating in all leaves during the 2-h feed was $^{14}$C-Suc (Fig. 4, C and D). Photosynthesis was greater and more $^{14}$C-Suc accumulated in leaves of plants that had been grown under moderate light (Fig. 4, C and D, solid line) than in those grown under low light (Fig. 4, C and D, dotted line). Specific activities of $^{14}$C-Suc increased rapidly in the leaves of the PK$_{c}^1$ plants during the 1st h of feed (Fig. 4E). The specific activities of $^{14}$C-Suc of plants grown under low light increased similarly. However, the specific activity of $^{14}$C-Suc was lower in the PK$_{c}^2$ leaves (Fig. 4F) in the PK$_{c}^1$ leaves (Fig. 4E). The specific activity of $^{14}$C-Suc in PK$_{c}^2$ leaves was slightly higher in the plants grown under low light. Nevertheless, taken together the data clearly show that in all cases the specific activity of the $^{14}$C-Suc was unchanged after 90 min (Fig. 4, E and F).

Export rates reported below (Fig. 5) were calculated from $^{14}$C retention and photosynthesis rates obtained between 90 and 120 min of the feed when it was assumed that the $^{14}$C-Suc was leaving the leaf as readily as it was being synthesized from newly fixed $^{14}$CO$_2$.

Estimates of Export Rates during Photosynthesis and $^{14}$C Partitioning during the Feed

The photosynthesis rates of plants grown under moderate light ranged from 14.5 to 16 $\mu$C m$^{-2}$ s$^{-1}$ (Figs. 3 and 5A). All lines grown under low light exhibited significantly lower leaf photosynthesis rates than plants grown under moderate light (Figs. 3 and 5B). The photosynthesis rates of plants grown in greenhouses during winter months were similar to those raised in growth chambers at the lower PPFD level (Fig. 5, B and C). The photosynthesis rates of plants grown in greenhouses during winter months were similar to those raised in growth chambers at the lower PPFD level (Fig. 5, B and C). The photosynthesis rates of plants grown in greenhouses during winter months were similar to those raised in growth chambers at the lower PPFD level (Fig. 5, B and C).

When leaves grown at the same light level were compared, there was no difference in the partitioning of label into starch and sugars. There appeared to be more label accumulated in starch in the plants growing in the greenhouse (Fig. 5, C and F), which reflected the fact that the immediate export rates were slightly lower in these plants. The patterns of $^{14}$C distribution among the ethanol-soluble

![Figure 3](image-url)  
Figure 3. Leaf photosynthetic light-response curves of PK$_{+1}$ and PK$_{c}^1$ tobacco plants. Net photosynthesis was measured when the first flower bud appeared on plants grown in growth chambers under 500 (A) or 100 (B) $\mu$mol m$^{-2}$ s$^{-1}$ PPFD. Photosynthesis was measured at 25°C ± 1°C. The CO$_2$ and O$_2$ levels were 35 Pa and 21 KPa, respectively. Each value represents the mean ± SE obtained with at least six leaves on six different plants.

![Figure 4](image-url)  
Figure 4. $^{14}$C-Labeling patterns of intact, attached source leaves of PK$_{+1}$ (A, C, and E) and PK$_{c}^1$ (B, D, and F) tobacco plants. A and B, Total $^{14}$CO$_2$ fixation, $^{14}$C retention, and $^{14}$C export of leaves of PK$_{+1}$ (A) and PK$_{c}^1$ (B) plants grown at 500 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD. Measurements were made during a 2-h $^{14}$CO$_2$ feed at 35 Pa CO$_2$, 25°C, and 1000 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD. Total C fixation (dashed line) was calculated from IR gas analyzer data. The $^{14}$C retention was monitored noninvasively with the GM detector (solid line) and invasively (●) following ethanol extraction. Export during steady-state $^{14}$CO$_2$ feeding (dotted line) was estimated as the difference between total fixation and $^{14}$C retention in the leaf. C and D, Accumulation of $^{14}$C-Suc in the source leaves of plants grown under low and moderate PPFD (i.e. 100 and 500 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD), respectively, but analyzed at a saturating PPFD for photosynthesis. E and F, Time course for changes in specific activities of $^{14}$C-Suc in the source leaves of plants grown under low and moderate PPFD levels, respectively. Each point represents the mean value (±SE) obtained with four leaves of four different plants.
pools (i.e. sugars, organic acids, and amino acids) were similar in leaves of the PKc2 and PKc1 lines (Fig. 5, G–I). For example based on Student’s t tests (not shown), there was no overall difference in the amount of 14C accumulated in organic acids in leaves of PKc2 and PKc1 plants at the end of the 2-h feed period. In all instances, 14C sugars accounted for the largest pool (i.e. 60%–75% of the ethanol-soluble 14C products). The major observations were that plants grown at 500 μmol m−2 s−1 PPFD had elevated photosynthesis rates and stored 35% to 40% more 14C than did plants grown at 100 μmol m−2 s−1 PPFD (Fig. 5, G and H). Previously fixed 14CO2 was primarily in the form of 14C-Suc and 14C-starch at the end of the feed periods (Figs. 4, C and D, and 5, A–C, G–I). The fate of the 14C products that accumulated during the 2-h labeling period was investigated further.

Leaf Dark Respiration and Nighttime Export of Labeled Assimilates

Figure 6 shows examples of traces obtained from GM detectors, which monitored the accumulation of 14C in leaves during the 2-h feeding period and the disappearance of label during the subsequent 14-h chase in the dark. Export and dark respiratory losses were variable during the chase. Although it was not determined whether the rapid decrease in radioactivity in the leaves at the beginning of the dark period was due to increased export or respiration, the overall pattern was similar in PKc+ leaves (Fig. 6, A and B) and PKc− (Fig. 6, C and D). During growth under moderate light, dark export of 14C (Fig. 7A) and respiratory 14CO2 evolution (Fig. 7D) were similar in leaves of all four lines. However, it is notable that during growth under low light the rates of 14C-labeled photosynthetic export in the dark were about 40% lower (Fig. 7B) and respiratory 14CO2 evolution were 40% greater (Fig. 7E) in the PKc− leaves as compared with the PKc+ controls.
Similarly, during winter months when natural PPFD levels are quite variable, leaves of PK\textsubscript{c}− plants raised in the greenhouse also exported less \textsuperscript{14}C (Fig. 7C) and released more \textsuperscript{14}CO\textsubscript{2} at night than did leaves of the PK\textsubscript{c}+ controls (Fig. 7F).

**DISCUSSION**

Leaves of the tobacco plants used in the present investigation were either deficient in PK\textsubscript{c} or they possessed wild-type levels of the enzyme (Fig. 1). PK activity in leaves is similar to those previously reported for leaves of the PK\textsubscript{c}− plants (Gottlob-McHugh et al., 1992; Knowles et al., 1998). Furthermore, antigenic staining of PK\textsubscript{c} was either absent or barely detectable on immunoblots of PK\textsubscript{c}− leaf extracts (Fig. 1). Residual PK activity of PK\textsubscript{c}− leaves has been attributed to PK\textsubscript{p} (Gottlob-McHugh et al., 1992). The transgenic tobacco deficient in PK\textsubscript{c} exhibited a marked reduction in root growth, which became much more pronounced during growth under low light (Knowles et al., 1998). The objective of this study was to investigate the possibility that primary photoassimilate export from the source leaves was somehow modified by the deficiency of leaf PK\textsubscript{c}.

The reduction in leaf PK\textsubscript{c} (Fig. 1) did not affect whole-plant net C gain (Fig. 2), leaf photosynthesis (Figs. 3–5), \textsuperscript{14}C partitioning in the leaf (Fig. 5G), or capacity of the leaf to export newly fixed CO\textsubscript{2} in the light or export stored \textsuperscript{14}C assimilates during a subsequent nighttime chase when the plants were grown at 500 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1} (Fig. 7A). These results are consistent with those of Gottlob-McHugh et al. (1992), who demonstrated that leaves of the PK\textsubscript{c}− transgenic grown under 400 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1} PPFD exhibited normal rates of photosynthetic O\textsubscript{2} evolution (and respiratory O\textsubscript{2} consumption). There were no differences between plant groups in the capacity of their leaves to assimilate CO\textsubscript{2} over a range of PPFD levels, including the two PPFD levels at which the plants were grown. Similarly, the rates of export of newly fixed C during a \textsuperscript{14}CO\textsubscript{2} feeding were identical. These data indicate that in source leaves the PK\textsubscript{c} is not an absolute prerequisite for (a) CO\textsubscript{2} fixation, (b) metabolism of \textsuperscript{14}C photoassimilates, or (c) phloem loading and export during photosynthesis (i.e. in the light). The major difference between the PK\textsubscript{c}− and the PK\textsubscript{c}+ plants, which would account for the delayed development and reduced root growth of the PK\textsubscript{c}− lines grown under low light, was the increased nighttime allocation of previously fixed C to respiration versus export (Fig. 7, B and E). The reduction in nighttime export could have a cumulative, negative influence on sink development.

At the time of appearance of the first flower bud, this organ functions as a strong sink for \textsuperscript{14}C assimilates (B. Grodzinski and J. Jiao, unpublished data). At this stage, about 55% to 60% of the \textsuperscript{14}CO\textsubscript{2} being fixed was being exported immediately (Fig. 5, D and E). Also, about 40% to 45% of the \textsuperscript{14}C photoassimilates remained in the leaf (Fig. 5, D and E). The \textsuperscript{14}C retained in the leaves of all plants was available for further metabolism and export during dark periods. The plants acclimatized to 500 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1} PPFD fixed more \textsuperscript{14}CO\textsubscript{2} and therefore retained more \textsuperscript{14}C at the end of the feed than did the plants cultivated under 100 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1} PPFD. Thus, there are two factors affecting mobilization and respiration rates during the dark chase period in the PK\textsubscript{c}− lines, which must be considered in these experiments. The first is that these plants were clearly deficient in PK\textsubscript{c} (Fig. 1). The second equally important consideration is that photosynthesis rates were lower and the pool sizes of \textsuperscript{14}C intermediates were correspondingly lower in all plants acclimatized to 500 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1} versus plants acclimatized to 100 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1} PPFD. It appears that the PK\textsubscript{c} step of cytosolic glycolysis can effectively be bypassed by alternative enzyme(s) if there are sufficient reserves of intermediates to drive other reaction sequences. In plants grown under low light there may be a higher, proportional respiratory cost associated with the translocation of \textsuperscript{14}C intermediates at night. The enzyme (or enzymes) being used to bypass PK\textsubscript{c} in the PK\textsubscript{c}− less leaves may not regulate glycolytic flux in support of dark respiration as effectively as PK\textsubscript{c}. Thus, the dark respiration of photoassimilates might be elevated, which could drain the pool of available Suc for phloem loading/translocation to sinks (hence, reducing export).

In cotton plants export in the dark was highly correlated with carbohydrate levels (i.e. starch) at the end of the photoperiod and with dark respiration rates (Hendrix and Grange, 1991). To some extent, \textsuperscript{14}CO\textsubscript{2} respiratory losses may reflect dark respiration rates in the leaves, which might be providing energy to sustain export in the dark. Nighttime export requires additional energy than in the light that can only be derived from stored photoassimilates (Giaquinta, 1972; Côté et al., 1992; Geiger and Servaites, 1972).
Thus, fine regulation of preexisting PKc bypass enzymes phosphatase or PEP carboxylase (Knowles et al., 1998). Bouma et al. (1995) calculated that as much as one-third of the C stored in starch could be lost through dark respiration in support of the energy requirements for photoassimilate mobilization and export. However, in the PKc− lines an increased respiration rate (i.e. 14CO2 production) may not reflect an increased rate of ATP production via oxidative phosphorylation. There may in fact have been a reduction in ATP production via oxidative phosphorylation. In the absence of normal levels of PKc some or all of the “enhanced” respiratory CO2 loss might occur via the nonphosphorylation (cyanide resistant) pathway of mitochondrial electron transport (involving the alternate oxidase). PKc regulation may be very important to the partitioning of electrons between phosphorylating and nonphosphorylating pathways of mitochondrial electron transport, since pyruvate is apparently the key activator of the alternative oxidase/CN-resistant pathway of mitochondrial respiration (Day et al., 1994). Thus, in PKc− leaves a depletion of stored assimilates via a bypass pathway coupled with a reduced supply of energy via oxidative phosphorylation for loading and translocation processes may effectively jeopardize the opportunity for the PKc− leaf tissue to mobilize carbohydrate reserves at night.

The PKc− leaves had higher rates of 14CO2 loss and exported less 14C during the dark than did PKc+ plants, but only during cultivation under low light when the total amount of 14C assimilates produced was reduced. The plants were at a similar stage of development and the relative rates of export during photosynthesis (i.e. export expressed as a percentage of photosynthesis) of these leaves were similar (Fig. 5, D and E). A primary difference between the PKc− plants grown at low versus moderate light was the amount of 14CO2 initially fixed during the feed (Fig. 5, A, B, G, and H). Further studies are required to distinguish how PKc and PKc bypass enzymes are regulated when plants are cultivated under very low irradiance. Our previous study revealed that, to compensate for the physical deficiency of leaf PKc, the PKc− leaves did not up-regulate the activities the PKc bypass enzymes PEP phosphatase or PEP carboxylase (Knowles et al., 1998). Thus, fine regulation of preexisting PKc bypass enzymes may allow the PKc− leaves to partially cope with PKc deficiency. Further studies are required to assess whether this is the case and, if so, whether it might be achieved by the “constitutive” phosphorylation of the leaf PEP carboxylase into its phosphorylated, more active (i.e. less malate inhibited) state.

Leaves of PKc+ and PKc− plants raised in a greenhouse during winter months demonstrated photosynthesis (Figs. 5C), dark export, and respiration characteristics (Figs. 7, C and F) that were similar to those of plants grown in chambers under low light (Figs. 5B and 7, B and E, respectively). Growing the test plants in a greenhouse during our winter months represents a different light-limited growth condition. The plants cultivated in the greenhouse were subject to wide fluctuations in PPFD during the photoperiod. Most plants in nature are exposed to varying levels of PPFD during their development. Diurnal transitions are obvious, but cloud cover, sun flecking, and mutual shading are also commonplace events influencing leaf metabolism and function (Boardman, 1977). Our results suggest that the presence of PKc and the operation of a “normal” glycolytic pathway are important in the production of energy needed for the mobilization of photoassimilates, when energy derived in the light from photosynthesis is not (directly) available, or when the levels of carbohydrates destined for export are too low to drive Suc synthesis, loading, and translocation processes required to meet sink demand.

In this study total nighttime export rates were not measured in all of the leaves in the canopy. Rather, the disappearance of label following a 2-h period of 14CO2 labeling during which a steady rate of photosynthesis was maintained. In sugar beet, following longer labeling periods (e.g. 6 h), it was shown that during the first 2 to 3 h in the dark the source of C for export was from the Suc pool that accumulated during the light period and that the concentration of Suc was an important factor controlling the rate of translocation during the subsequent dark period (Geiger and Batey, 1967). Thereafter, starch was mobilized to form Suc for translocation. In tobacco 14C-Suc accumulated during the feed period (Fig. 4, C and D) and likely served as a source of exported 14C during the nighttime chase (Fig. 6). In the tobacco leaves acclimatized to growth at either the low or the moderate light, the fastest rate of disappearance of previously fixed 14C occurred during the first hours of the dark period (Figs. 6 and 7). In this study nighttime export would be underestimated in all of the lines. A reduced export rate in the PKc− lines during the dark chase period means that the actual reduction in translocation of photoassimilates among these plants was probably much greater than that reported here.

Taken together, our results underscore the problems of raising transgenic plants in different growth conditions and/or relying solely on data derived during growth under one light condition. To our knowledge, this is the first study in which the expression in leaves of a glycolytic enzyme was altered and photosynthesize export rates were measured both in the light and in the dark. Selected modification of other reactions associated more directly with C transport and phloem-loading processes do alter translocation (Lerchl et al., 1995; Geigenberger et al., 1996; Hattenbach et al., 1997; Hausler et al., 1998). A major difference noted in the transgenic plants deficient in leaf PKc was the reciprocal effect on C export and dark respiration in low-light-grown plants that were not assimilating CO2 at a fast rate. This was not observed when the PKc− plants were grown at a moderate light level. Further insight into the role of PKc and glycolysis in maintaining homeostasis in source leaves may be provided by challenging transgenic plants with growth conditions in which nutrients, as well as light conditions, are varied.

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LITERATURE CITED


