Expression of Maize Phosphoenolpyruvate Carboxylase in Transgenic Tobacco

Effects on Biochemistry and Physiology

Richard L. Hudspeth*, John W. Grula, Ziyu Dai, Gerald E. Edwards, and Maurice S. B. Ku

ABSTRACT

The expression of maize (Zea mays) phosphoenolpyruvate carboxylase (PPC) gene constructions was studied in transgenic tobacco plants (Nicotiana tabacum). Where transcription was under the control of a maize PPC gene promoter, a low level of aberrantly large PPC transcript was detected. Analysis of this PPC transcript indicated that transcription initiation occurs upstream of the normal site. Despite the aberrant transcription initiation, expression of the PPC transcript was still light-regulated. Higher levels of maize PPC transcript of the correct size were obtained with a chimeric gene construction containing a tobacco (Nicotiana plumbaginifolia) chlorophyll a/b binding protein gene promoter. The PPC activities in the leaves of these transgenic plants were up to twofold higher than those of nontransformed plants. Two forms of PPC with different kinetic properties were identified in leaf extracts of the transgenic plants: one form with a high apparent Kms for phosphoenolpyruvate (maize isozyme), and a second form exhibiting a low apparent Kms (tobacco isozyme). Biochemical analyses of these plants indicated that the transgenic plants had significantly elevated levels of titratable acidity and malic acid. These biochemical differences did not produce any significant physiological changes with respect to photosynthetic rate or CO2 compensation point.

C4 plants possess a number of desirable agronomic characteristics. These include high photosynthetic capacity, growth rate, and nutrient and water use efficiency. These traits are especially apparent under high light and temperature conditions (7). Evaluation of the potential to transfer C4 characteristics into C3 plants by conventional breeding has been an active research area in plant biology (1, 4, 26). However, hybridization between C3 and C4 plants has been successful only in a few plant genera and none of them are crop species. In addition, most of the hybrids exhibit infertility due to irregular chromosome pairing or other genetic barriers. An earlier study on hybrids between C3 and C4 Atriplex species (26) concluded that the inheritance of the biochemistry of C4 photosynthesis and of “Kranz” anatomy are not closely linked, and the segregation of a hybrid possessing complete and fully coordinated C4 photosynthesis is a rare event. Thus, employing traditional breeding methods to incorporate C4 traits into C3 crops will be difficult if not impossible.

With the development of plant genetic engineering, transfer of foreign genes into plants has become commonplace, providing a new approach to altering plant traits. A genetic engineering approach to the transfer of C4 traits into C3 crops may bypass the problems that are encountered with traditional breeding.

PPC performs an essential role in leaves of C4 plants, catalyzing the primary fixation of atmospheric carbon (for a review see ref. 7). The reaction catalyzed by PPC is the first step of the C4 pathway, a process that ultimately reduces the energy loss by photorespiration. In the C4 plant maize (Zea mays), carbon fixed by PPC is transported as malate from mesophyll cells to bundle sheath cells. The malate is decarboxylated in the chloroplasts of bundle sheath cells, elevating the ratio of CO2 to O2. The elevated level of CO2 in the bundle sheath chloroplast overcomes the wasteful process of photorespiration caused by the oxygenase activity of RuBPC (7, 15).

Although PPC is best known for its role in C4 photosynthesis, isozymes of PPC are nonphotosynthetic in both C3 and C4 plant species (25). Previously, we and others have characterized the maize PPC gene (Ppcl) encoding the isozyme involved in C4 photosynthesis (11, 21). In this report, we describe the expression of three different maize Ppcl gene constructions in transgenic tobacco and how this affects the biochemistry and physiology of these plants.

MATERIALS AND METHODS

Plasmid Constructions and Transformation of Tobacco

Three different plasmids were constructed containing maize (Zea mays) Ppcl sequence. These constructions were designated BVI-9500, BVI-7000, and BVI-6400 (see Fig. 1A). The insert of BVI-9500 contains the intact Ppcl gene expressed in maize leaves plus about 2.0 kb of upstream and 1.8 kb of downstream sequence. The 9.5 kb HindIII fragment contain-

---

1 This work was supported by the J.G. Boswell Company (Los Angeles).

2 Abbreviations: PPC, phosphoenolpyruvate carboxylase; RuBPC, ribulose 1,5-bisphosphate carboxylase; bp, base pairs; kb, kilobase pairs.
ing the maize Ppc1 gene was isolated from the genomic clone H1\(\lambda\)14 (11).

The insert of the plasmid BVI-7000 is a 7.0 kb HindIII fragment containing a maize Ppc1 gene without introns. This construction, also containing about 2.0 kb of upstream and 1.8 kb of downstream sequence, was made by the ligation of Ppc1 genomic and cDNA sequence. The 5'-portion of the construction was obtained by the ligation of a 2.4 kb HindIII-Apal fragment contained in H1\(\lambda\)14 to a 350 bp Apal-BamHI fragment isolated from the Ppc1 cDNA clone, pPEPC4 (11). Similarly, the 3'-portion of the construction was derived from the ligation of a 1.8 kb Nsil-HindIII fragment contained in H1\(\lambda\)14 to a 2.4 kb BamHI-Nsil fragment from pPEPC4.

The insert of the plasmid BVI-6400 contains a chimeric gene consisting of Nicotiana plumbaginifolia Chl a/b binding protein (Cab) gene 5'- and 3'-flanking regions fused to Ppc1 coding sequence from pPEPC4. The 6.4 kb HindIII insert of BVI-6400 includes 1.6 kb of 5'-flanking sequence and the entire 3'-nontranslated sequence of the tobacco Cab-E gene (3). A translational fusion of the Cab-E gene promoter and the Ppc1 cDNA sequence was made at the start codon (ATG) of both sequences. The 3'-end fusion of the Cab-E gene with the Ppc1 gene was made such that BVI-6400 contains 120 bp of Ppc1 3'-nontranslated sequence followed by 30 bp of Cab-E gene 3'-nontranslated sequence and 1.7 kb of Cab-E gene 3'-flanking sequence.

To construct BVI-6400, M13 subclones were prepared from 2.0 and 2.3 kb EcoRI-PstI fragments isolated from the tobacco Cab-E gene clone AB1 (a gift from Anthony Cashmore, University of Pennsylvania). Restriction sites were introduced into the Cab-E gene subclones by site-directed mutagenesis (18). A 20 bp oligonucleotide (AGAAGCAGCCATGGT-AGGAG) was used to create an Ncol site in the 2.0 kb EcoRI-PstI insert. Similarly, a 30 bp oligonucleotide (GGAAGTGGAAGTCATTAAATACGAGTCTCT) was used to generate an NsiI site in the 2.3 kb subclone. The 5'-portion of the construction was obtained by the ligation of a 1.5 kb HindIII-Ncol fragment, isolated from the mutated 2.0 kb subclone, with a 1.2 kb Ncol-EcoRI fragment contained in pPEPC4. The 3'-portion of the construction was obtained by the ligation of a 1.7 kb NsiI-HindIII fragment, isolated from the mutated 2.3 kb Cab-E gene subclone, to a 2.0 kb EcoRI-NsiI fragment contained in pPEPC4.

The vector plasmids (BVI-9500, BVI-7000, and BVI-6400) used for tobacco transformations were generated by the ligation of HindIII fragments containing the different gene constructions into the unique HindIII site contained in the binary vector plasmid BVI (23). The integrity of all constructions was checked by restriction mapping (see Fig. 1A). Transformations of Agrobacterium tumefaciens strain LBA4404 (8) with the different vector plasmids were performed as described by Holsters et al. (9) and the cells harboring the vector plasmids were selected on gentamycin (50 \(\mu\)g/mL). Tobacco (Nicotiana tabacum; var SR1) leaf discs were infected with Agrobacterium strains containing each of the vector plasmids, and kanamycin-resistant plants were regenerated from independently isolated (nonclonal) transformed shoots as described by Horsch et al. (10).

**Growth Conditions**

Plants were established from stem cuttings and grown in a chamber with 16 h of light at 30°C (50–60% relative humidity) and 8 h of darkness at 18°C (60–70% relative humidity). The light intensity, provided by high intensity metal halide lamps, was about 600 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) at the plant canopy. Plants were fertilized twice a week with commercial fertilizer and supplemented with micronutrients. Young and newly expanded leaves (usually the third or fourth leaves from the apex) from 6- to 8-week-old-plants were used for all experiments.

**Isolation and Labeling of Nucleic Acids**

Total tobacco DNA was extracted from leaves as described by Dellaporta et al. (5). Plasmid DNAs were prepared by the protocol of Krieg and Melton (14) and probes for gel blot hybridization were prepared by nick-translation (19). Total RNA was extracted from tobacco leaves using the protocol described in Kühlemeyer et al. (17) and the poly(A)\(^+\) fraction was isolated by one pass over oligo(dT) cellulose (19).
Western Immunoblotting

Thirty or 60 μg of soluble leaf protein, extracted as described above, was subjected to SDS-PAGE on 12% (w/v) polyacrylamide gel containing 0.2% (w/v) SDS. After electrophoresis, the polypeptides were transferred from the gel to a nitrocellulose membrane. The membrane was probed with antibody raised against purified maize PPC and alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G antibody (20).

Metabolite Extraction and PPC Assays

Leaf titratable acidity and malate content were measured after a period of 8 h in the dark (predawn) and after a period of 16 h in the light (predusk). Extraction of the metabolites and assays of acidity and malate were as described by Ku et al. (16).

Measurements of Photosynthesis and O2 Inhibition of Photosynthesis

Photosynthesis rates of individual leaves were determined in an open system using an ADC infrared gas analyzer and a Bingham Interspace model BI-2 controller system. The CO2 concentration in the leaf chamber was maintained at 345 μbar using a null balance system by the controller, and leaf temperature was maintained at 30°C. The irradiance during photosynthesis measurements was 1000 μmol m−2 s−1. To determine the sensitivity of photosynthesis to O2, photosynthesis was measured at O2 concentrations between 21 and 2%.

CO2 Compensation Point Measurements

The photosynthetic CO2 compensation point was measured using an ADC infrared gas analyzer in an absolute mode. A newly expanded leaf was sealed into a Plexiglas chamber under an irradiance of 1000 μmol m−2 s−1. The leaf temperature was maintained at 30°C using a peltier cooling plate; a small fan mixed the air inside the chamber. CO2 concentration inside the chamber was continuously monitored and recorded by circulating the air through the CO2 analyzer. The compensation point was determined when the CO2 concentration

Figure 3. Northern blot of leaf RNA from tobacco plants transformed by BVI-6400 and hybridized with a Ppc1 cDNA probe (pPEPC4; 11). Lane 1, 0.3 μg of total leaf RNA from maize (control); lane 2, 10 μg of total leaf RNA from nontransformed tobacco (control); lane 3, 10 μg of total leaf RNA from the transgenic tobacco plant BVI-6400 (1) grown in the dark; lanes 4 to 7, 10 μg of total leaf RNA from transgenic tobacco plants BVI-6400 (1–4) grown in the light.

Gel Blot Hybridization Procedures

Gel blot hybridization was used to detect the presence of the Ppc1 gene sequence in transformed tobacco plants. Total DNA (10 μg) was digested with HindIII, separated by electrophoresis through a 0.8% (w/v) agarose gel, and transferred to nitrocellulose (29). Electrophoresis of RNA was through agarose gels containing 6% (v/v) formaldehyde as described by Maniatis et al. (19). Hybridizations to 32P-labeled probes were performed as described by Klessig and Berry (13).

Enzyme Extraction and Assay

About 0.5 g of illuminated leaf tissue was harvested in the middle of the day, quickly frozen in liquid nitrogen, and ground into fine powder using a mortar and pestle. Ten volumes of extraction medium was added and grinding continued until maceration was complete. The extraction medium contained 50 mM Hepes-KOH, pH 8.0, 10 mM MgCl2, 1 mM EDTA, 10 mM DTT, 10% (w/v) insoluble PVP, 12.5% (v/v) glycerol, 10 μM leupeptin, and 1 mM PMSF. The crude extract was filtered through one layer of Miracloth (Calbiochem, La Jolla, CA) and aliquots were taken for Chl and protein determinations. The filtrate was centrifuged at 15,000g for 5 min at 4°C and the supernatant was rapidly desalted by centrifugation through a small Sephadex G-25 column. The column was preequilibrated with the extraction medium without PVP. The eluate was used for enzyme assay immediately.

Enzymes were assayed spectrophotometrically at 30°C in a volume of 1 mL as previously described by Edwards and Jenkins (6).
Transformation and Selection of Plants

Three vector plasmids (Fig. 1A) were used for plant transformations. BVI-9500 includes the entire PPC gene (Ppcl) expressed in maize leaves plus 2.0 kb of 5'-flanking and 1.8 kb of 3'-flanking sequence. BVI-7000 contains a Ppcl gene lacking intron sequence, and was constructed to study the effect of introns on transcript level. BVI-6400 was constructed with promoter and terminator sequences from the tobacco Cab-E gene because monocot promoter and terminator sequences can function poorly in dicots (12).

Multiple independent transformants were selected on kanamycin and resistant shoots were regenerated into plants. DNA isolated from regenerant plants was hybridized with a Ppcl cDNA probe (pPEPC; see ref. 11), and regenerants with unaltered gene constructions were identified by the size of the hybridizing HindIII fragment (Fig. 1B).

Analysis of Maize PPC Transcripts in Transgenic Plants

RNA extracted from transformants was probed to determine the steady-state level of maize PPC transcript. As shown in Figure 2A, plants transformed with plasmids BVI-9500 and BVI-7000 had low levels of maize PPC transcript. The levels were two to three orders of magnitude lower than the level in maize leaves, based on the relative intensities of the northern blot hybridizations. The maize PPC transcript levels in plants transformed by BVI-9500 were about the same as in plants transformed by BVI-7000, which indicates the Ppcl gene introns have no major influence on transcript level. This result is not surprising, although the introns of some genes are known to affect transcript levels significantly (2).

The estimated size of the maize PPC transcript in the plants transformed with BVI-9500 and BVI-7000 was 4.2 kb, which is 700 bp larger than the mRNA transcribed from the same gene in maize. Interestingly, it has been shown that a transcript of the correct size (3.4 kb) is expressed in transgenic tobacco plants containing an intact PPC gene from sorghum (30). The abnormally large maize PPC transcript could result from inefficient intron processing, inaccurate transcription initiation, or inaccurate transcription termination. Altered termination and splicing have been observed with other heterologous plant genes (12). The first possibility can be excluded because BVI-7000 transformants exhibited the same 4.2 kb transcript size as BVI-9500 transformants. Furthermore, an intron III-specific probe from the Ppcl gene did not hybridize with leaf RNA from the transgenic plants (data not shown).

Table I. Enzymatic Properties of Plant Leaf Extracts

<table>
<thead>
<tr>
<th>Plant Material</th>
<th>Activity</th>
<th>Kinetic Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RuBPC*</td>
<td>K&lt;sub&gt;v&lt;/sub&gt;(PEP)</td>
</tr>
<tr>
<td></td>
<td>μmol/mg Chl h</td>
<td>+G6P</td>
</tr>
<tr>
<td>Maize</td>
<td>174</td>
<td>2.222</td>
</tr>
<tr>
<td>Tobacco</td>
<td></td>
<td>1.175</td>
</tr>
<tr>
<td>SR1 (control)</td>
<td>316</td>
<td>0.025</td>
</tr>
<tr>
<td>BVI-6400(1)</td>
<td>324</td>
<td>0.024</td>
</tr>
<tr>
<td>BVI-6400(2)</td>
<td>280</td>
<td>0.125</td>
</tr>
<tr>
<td>BVI-6400(3)</td>
<td>276</td>
<td>0.022</td>
</tr>
<tr>
<td>BVI-6400(4)</td>
<td>143</td>
<td>0.029</td>
</tr>
</tbody>
</table>

* The data presented are the means of two replicates. For each plant, the values differ by less than 10%.

** PPC was assayed using 2 to 4 μmol PEP (saturated) in the absence of glucose-6-phosphate (G6P). The data presented are the means of four replicates (± se). The difference between the control and the transgenic plant data sets is significant at the 99% level by Tukey’s test (28).

---

Downloaded on June 2, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.
The levels of maize PPC transcript in plants transformed by BVI-6400 were one to two orders of magnitude lower than the level in maize leaves (Fig. 3). These PPC transcript levels are substantially higher than the levels in the BVI-9500 and BVI-7000 transformatns. There could be several reasons for this difference, although it probably reflects the relative strengths of the Ppc1 and Cab-E gene promoters in tobacco. In addition, the PPC transcripts in BVI-6400 transformants comigrate with the PPC transcript in maize (3.5 kb).

The levels of maize PPC transcript in tobacco plants transformed by BVI-6400 are reduced in plants grown in the dark (Fig. 3). This result was expected, as transcription directed by the Cab-E gene promoter is known to be light-regulated (27). As shown in Figure 2, the level of maize PPC transcript in BVI-9500 and BVI-7000 transformants is also light-regulated. These results are consistent with the findings of Matsuoka and Sanada (22), who demonstrated the light-inducible expression of β-glucuronidase in leaves of tobacco plants transformed with a chimeric gene under the control of the Ppc1 gene promoter. Therefore, the mechanism regulating the light-inducible expression of the Ppc1 gene is conserved in monocotyledonous and dicotyledonous plants. Furthermore, this mechanism is unperturbed by the inaccurate transcription initiation of the Ppc1 gene in transgenic tobacco.

**Biochemical and Physiological Analyses of Transgenic Tobacco**

Because BVI-6400 transformants had the highest level of maize PPC transcript, these plants were selected for various biochemical and physiological analyses. To determine if the BVI-6400 transformants had elevated levels of PPC, a western blot of leaf proteins from BVI-6400 transformants was probed with a maize PPC antibody (Fig. 4). Although the maize PPC antibody cross-reacts with the C3 form of PPC in the tobacco control, the blot indicates that the transgenic plants have increased amounts of PPC. However the levels are still low relative to that in maize leaves.

The increased level of PPC in the leaves of BVI-6400 transformants was confirmed by measurements of enzyme activity. The four transgenic plants tested had about twofold higher PPC activity on a Chl basis than the control (Table I). On the other hand, the activity of RuBPC in the transgenic plants was the same or lower than in the control.

The differences in affinity for substrate and activation by metabolites on PPC from C3 and C4 plants are well established (24). As expected, PPC from the control tobacco had a low $K_a_{PEP}$ of 25 μM, whereas the control maize PPC exhibited a high $K_{a_{PEP}}$ of 2,222 μM (Fig. 5). In addition, the metabolite glucose-6-phosphate increased the maximum activity of the tobacco enzyme without changing its affinity for the substrate, but increased the affinity for the substrate of the maize enzyme without changing its maximum activity (Table I). These different kinetic properties were used to detect the presence of two different PPC isoforms in the transgenic tobacco plants. In the transgenic plants tested, two kinetic forms of PPC were detected (Fig. 5, Table I). One corresponds to the tobacco isoform (low $K_m$ form) and the other corresponds to the maize isoform (high $K_m$ form). Apparent $K_m_{PEP}$ values were determined from the nonlinear reciprocal plots of the transgenic PPC activity.

Although there was also no hybridization with a probe for 3'-flanking sequence (data not shown), a probe including the sequence 133 to 1219 bp upstream of the transcription start site (11) did hybridize with the 4.2 kb transcript (Fig. 2B). Therefore, the aberrantly large maize PPC transcript of the transgenic plants can be explained by transcription initiation in 5'-flanking sequence. To our knowledge, this example of inaccurate transcription initiation of a foreign gene in plants is novel, although this phenomenon has been observed in yeast (27).

---

**Figure 5.** Double reciprocal plots of enzyme activity (μmol/mg Chl·h) as a function of PEP concentration (mM) for PPC extracted from leaves of (A) tobacco, (B) maize, and (C) transgenic tobacco [BVI-6400 (3)]. Enzyme activity was measured in the absence (○) or presence (△) of 5 mM glucose-6-phosphate. The kinetic values are listed in Table I.
plants tested. The apparent $K_m$ values for the maize isoform in extracts of the transgenic plants are considerably lower than the $K_m$ for PPC obtained from maize leaves (see Table I). This is because the $K_m$ values for the maize isoform were greatly influenced by the activity of the low $K_m$ (tobacco) isoform in the extract. A higher apparent $K_m$(PEP) value for the maize isoform would result from a higher relative amount of maize PPC in the transgenic plant. Based on this analysis, the transformant BVI-6400 (4) probably has the highest level of maize PPC activity among the transgenic plants tested. The differential effects of glucose-6-phosphate on the kinetic properties of the enzyme in the transgenic plants is also consistent with the presence of two PPC isoforms (Table I).

Higher PPC activity could elevate the level of organic acids if they are not further metabolized. We measured the levels of total titratable acid and malic acid in leaves of the transgenic plants to explore this possibility. In general, the transgenic plants had significantly higher leaf acidity and malate content than the control plants, in either light or darkness (Table II). Among the transgenic plants, BVI-6400 (3) and (4) accumulated the most malate and acidity during the light period. The highest level of malic acid was in leaves of BVI-6400 (4). The level of malic acid in this plant was over twice that of the control. Furthermore, it appears that BVI-6400 (4) does not reduce malate and the associated acidity at night to a level comparable to that of the other plants (Table II).

These results suggest that BVI-6400 (3) and (4) have more CO$_2$ fixed through the maize PPC. However, the extra CO$_2$ fixed by the maize PPC is probably small relative to the total CO$_2$ assimilation. For example, BVI-6400 (3) accumulated 4.5 $\mu$mol malate/mg Chl more than the control plant in a light period of 16 h (Table II), which translates into a net accumulation rate of 0.28 $\mu$mol/mg Chl-h. This tobacco plant has rates of CO$_2$ fixation of 148 $\mu$mol/mg Chl-h (Table III). Hence, depending on the degree to which the malate is metabolized, the contribution of maize PPC to total CO$_2$ fixation in these transgenic plants is a minimum of about 0.2% and probably not much higher than a few percent. Therefore, relative to C$_4$ plants the contribution of PPC to total CO$_2$ assimilation in the transgenic plants is still low.

Because the accumulation of organic acids in transgenic tobacco might indicate a change in metabolism, we undertook several photosynthetic measurements. As shown in Table III, measurements of the CO$_2$ compensation point indicated that there was no effect on the rate of photorespiration. Likewise, no differences were found in the sensitivity of photosynthesis to O$_2$ between the control and the transgenic tobacco plants (data not shown). The rate of photosynthesis for BVI-6400 (1), (2), and (3) was within experimental error of the rate for the control plant. However, BVI-6400 (4) had a significantly lower rate of photosynthesis, which is consistent with its lower RuBPC activity (Table I). Also consistent with a lower photosynthetic rate, BVI-6400 (4) appeared to grow more slowly than the other plants. The high acidity in the leaves of BVI-6400 (4) may have impaired normal leaf metabolism. Alternatively, the growth and photosynthesis characteristics of BVI-6400 (4) could be due to the somoclonal variation that is sometimes observed among regenerated plants.

In summary, the transgenic tobacco plants in this study perform the carboxylation step of the C$_4$ pathway, although at much lower levels than C$_4$ plants. The increased malate content of the transgenic plants suggests that they have limited

---

**Table II. Total Titratable Acidity and Malic Acid in Leaf Tissues**

The data presented for SR1 (control) are the means of four replicates ($\pm$ SE). Data for the transgenic plants BVI-6400(1–3) and BVI-6400(4) are the means of two and three replicates, respectively. The difference between the control and the transgenic plant data sets is significant at the 95% level (by Tukey’s test; see ref. 28) for both the total titratable acidity and the malic acid content.

<table>
<thead>
<tr>
<th>Plant Material</th>
<th>Total Titratable Acidity</th>
<th>Malic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predawn</td>
<td>Predusk</td>
</tr>
<tr>
<td>Maize</td>
<td>43.0</td>
<td>54.3</td>
</tr>
<tr>
<td>Tobacco</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR1 (control)</td>
<td>31.8 ± 6.0</td>
<td>42.0 ± 7.0</td>
</tr>
<tr>
<td>BVI-6400(1)</td>
<td>40.5</td>
<td>52.4</td>
</tr>
<tr>
<td>BVI-6400(2)</td>
<td>41.0</td>
<td>56.5</td>
</tr>
<tr>
<td>BVI-6400(3)</td>
<td>44.2</td>
<td>61.0</td>
</tr>
<tr>
<td>BVI-6400(4)</td>
<td>88.6</td>
<td>108.5</td>
</tr>
</tbody>
</table>

---

**Table III. Photosynthesis Rate and CO$_2$ Compensation Point of Intact Leaves**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Photosynthesis Rate$^*$</th>
<th>CO$_2$ Compensation Point$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$mol m$^{-2}$ s$^{-1}$</td>
<td>$\mu$mol/mg Chl-h</td>
</tr>
<tr>
<td>Maize</td>
<td>33.1 ± 0.7</td>
<td>249 ± 5</td>
</tr>
<tr>
<td>Tobacco</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR1 (control)</td>
<td>25.8 ± 1.7</td>
<td>171 ± 11</td>
</tr>
<tr>
<td>BVI-6400(1)</td>
<td>23.4 ± 2.5</td>
<td>163 ± 17</td>
</tr>
<tr>
<td>BVI-6400(2)</td>
<td>23.7 ± 1.0</td>
<td>159 ± 7</td>
</tr>
<tr>
<td>BVI-6400(3)</td>
<td>25.0 ± 1.5</td>
<td>148 ± 10</td>
</tr>
<tr>
<td>BVI-6400(4)</td>
<td>19.4 ± 2.5</td>
<td>137 ± 18</td>
</tr>
</tbody>
</table>

$^*$ Photosynthesis rates were measured at 30°C, 345 $\mu$bar CO$_2$, 21% O$_2$, and 1000 $\mu$mol quanta/m$^2$ s. Data presented are the means of 6–10 measurements ($\pm$ SE). $^b$ CO$_2$ compensation points were measured at 30°C, 21% O$_2$, and 1000 $\mu$mol quanta m$^{-2}$ s$^{-1}$. Data presented are the means of three measurements ($\pm$ SE).
capacity to utilize malate. Whether this increase in malate could be used as a carbon source for the Calvin cycle, particularly under limiting CO₂, might be evaluated by producing transgenic plants that also express the C₄ form of the chloroplastic NADP-malic enzyme.

ACKNOWLEDGMENTS

We are grateful to Y.S. Tai and C. Chu for their help in the biochemical analyses of the transgenic plants and to J.W. Pellow for his assistance in the tobacco transformations. We also thank A.J.M. and M.A. Matzke for the use of the binary vector plasmid BVI and A.R. Cashmore for his gift of the tobacco Cab-E gene clone AB1.

LITERATURE CITED

12. Keith B, Chua N-H (1986) Monocot and dicot pre-mRNAs are processed with different efficiencies in transgenic tobacco. EMBO J 5: 2419–2425