Promotive Effect of Low Concentrations of NaHSO₃ on Photophosphorylation and Photosynthesis in Phosphoenolpyruvate Carboxylase Transgenic Rice Leaves

Ben-Hua JI¹, Hong-He TAN¹, Rong ZHOU¹, De-Mao JIAO*² and Yun-Gang SHEN³

¹. College of Life Sciences, Nantong University, Nantong 226007, China; 2. Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China; 3. Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences, Shanghai 200032, China

Abstract: Spraying a 1–2 mmol/L solution of NaHSO₃ on the leaves of wild-type rice (Oryza sativa L.) Kitaake (WT), phosphoenolpyruvate carboxylase (PEPC) transgenic (PC) rice and PEPC+phosphate dikinase (PPDK) transgenic rice (PC+PK), in which the germplasm was transformed with wild-type Kitaake as the gene receptor, resulted in an enhancement of the net photosynthetic rate by 23.0%, 28.8%, and 34.4%, respectively, for more than 3 d. It was also observed that NaHSO₃ application caused an increase in the ATP content in leaves. Spraying PMS (a cofactor catalysing the photophosphorylation cycle) and NaHSO₃ separately or together on leaves resulted in an increase in photosynthesis with all treatments. There was no additional effect on photosynthetic rate when the mixture was applied, suggesting that the mechanism by which NaHSO₃ promotes photosynthesis is similar to the mechanism by which PMS acts and that both of compounds enhanced the supply of ATP. After spraying a solution of NaHSO₃ on leaves, compared with the WT Kitaake rice, a greater enhancement of net photosynthetic rate was observed in PEPC transgenic (PC) and PEPC+PPDK transgenic (PC+PK) rice, with the greatest increase being observed in the latter group. Therefore ATP supply may become the limiting factor that concentrates CO₂ in rice leaves transformed with an exogenous PEPC gene and exogenous PEPC+PPDK genes.

Key words: NaHSO₃; phosphoenolpyruvate carboxylase transgenic rice; photophosphorylation; photosynthesis.

The advent of genetic engineering in crop plants has recently resulted in attempts to introduce C₄ characteristics into C₃ plants. In rice, high expression of maize phosphoenolpyruvate carboxylase (PEPC) was achieved using the complete maize PEPC gene, including exons and introns, as well as its own promoter, for transformation and this resulted in a 110-fold increase in PEPC activity measured in vitro (Ku et al. 1999). Then a high expression of the double gene PEPC and phosphate dikinase (PPDK) was obtained (Ku et al. 2000). Relative to wild-type (WT) rice, PEPC transgenic rice (PC) exhibits a higher photosynthetic rate and characteristics of tolerance to photo-oxidation under strong light and higher temperatures (Jiao et al. 2001). Further studies demonstrated that this was caused by increasing the CO₂-concentrating capacity in photosynthetic cells (Huang et al. 2002; Jiao et al. 2003) and that there was a limited photosynthetic C₄ microcycle in the leaves of C₃ rice plants, but that insertion of an exogenous PEPC gene enhanced the existing, and limited, photosynthetic C₄ microcycle in PC transgenic rice (Ji et al. 2004). Sage (2002) reported that
**Borszczowia aralocaspica** and **Bienertia cycloptera** possess primitive types of C₄ photosynthesis without Kranz anatomy, suggesting that a primitive type of C₄ photosynthesis can run in single mesophyll cells. In particular, the carboxylation of phosphoenolpyruvate (PEP) by PEP carboxylase (PEPCase) occurs in the cytoplasm to form oxaloacetic acid (OAA), which is then converted to another C₄ acid (malate or aspartate). The C₄ acid diffuses into the chloroplasts and is decarboxylated in the chloroplast by a decarboxylase enzyme, such as NADP-malic enzyme (NADP-ME). The CO₂ released is fixed by Rubisco in the Benson-Calvin cycle. The C₃ acid released by the decarboxylation step returns to the cytoplasm, where it is phosphorylated by a pyruvate, namely PPDK, to produce PEP; this could be achieved at the expense of ATP for CO₂ transferred. The evidence from **B. aralocaspica** and **B. cycloptera**, that the C₄ system can function without Kranz anatomy, increasing the likelihood that a minimalist system could be realized in rice. More ATP is needed in the C₄ photosynthetic pathway than in the C₃ pathway, and, thus, it is possible that the ATP supply may become the limiting factor to further increasing the photosynthetic rate in transgenic rice. Studies by Wang et al. (2000) validated that low concentrations of extraneous NaHSO₃ had a promotive effect on photosynthesis by enhancing cyclic photophosphorylation in rice. In addition, photosystem (PS) is more stable than PSII during strong light treatment and because PSI photoinhibition has seldom been observed in vivo (Heber and Walker 1992), the major site of photoinhibition has been assumed to be PSII. Cyclic photophosphorylation is a process that has been suggested to play an important role in protecting against photo-oxidative stress (Fork and Herbert 1993). The aim of the present study was to explore the mechanisms, in rice leaves treated with lower concentrations of NaHSO₃, by which photoinhibitory damage was alleviated by increasing cyclic electron transport and how limited C₄ photosynthesis could be enhanced by supplying extra ATP.

## 1 Materials and Methods

### 1.1 Experimental materials and NaHSO₃ treatment

Untransformed WT rice (**Oryza sativa** L. subsp. **japonica** Kitaake), PC and PEPC+PPDK (PC+PK) transgenic rice, which were transformed with WT Kitaake as the gene receptor by Ku et al. (1999), were used in the present study. Seeds were germinated directly in soil and plants were grown in 4-L pots and maintained in a naturally illuminated netted outdoor room at Jiangsu Academy of Agricultural Sciences (Nanjing, China). There were five hills per pot and one seedling for each hill. The entire period of growth of the transgenic rice sown in the first 10 d period of May was approximately 90 d. The average temperature varied from 21 to 27 °C and the diurnal temperature difference was from 7.1 to 8.7 °C. Seedlings were watered and fertilized regularly during the growth period. The maximum photosynthetically active radiation (PAR) at noon on a sunny day in Nanjing during the period July-August was approximately 1 800 µmol·m⁻²·s⁻¹. The aforementioned rice material was used in assays of the photosynthetic oxygen evolution of chloroplasts and leaf discs, CO₂ exchange rates and chlorophyll fluorescence parameters of rice leaves. Experiments were performed at the milk-ripening stage of the rice by using fully expanded flag leaves. Bilaterally symmetrical rice flag leaves were selected. After removal of the middle vein, one half was sprayed with distilled water (as a control) and the other half was treated with NaHSO₃ solution in the late afternoon of fine days.

### 1.2 Measurement of photosynthetic rate

The net photosynthetic rate (Pn), stomatal conductance (Gs), and intercellular CO₂ concentration (Ci) of rice leaves were measured with a portable photosynthetic gas exchange analyzer system (TPS–1; Hansatech, Instruments Ltd, UK) under sunlight or a white light with a PFD of 1 000 µmol·m⁻²·s⁻¹ (quartz halogen lamp), which passed a layer of 12 cm water as a heat filter. Measurements were made at approximately 25 °C. Leaf discs with an area of approximately 1.5
mm × 1.5 mm were obtained from leaves of rice grown in the outdoor netted room and were submerged into buffer containing 0.4 mmol/L NaHCO₃ (pH 7.5). Photosynthetic oxygen evolution rates of leaf discs were determined using the Oxylab system (Oxylab system, liquid phase; Hansatech) under a PFD of 1 000 µmol·m⁻²·s⁻¹. Experiments were repeated five times.

1.3 Measurement of ATP content

Measurements of ATP content were performed according to the methods of Wang (1985), with slight modification. Bilaterally symmetrical rice flag leaves were selected. After removal of the middle vein, one half was sprayed with distilled water (as a control) and the other half was treated with 1.5 mmol/L NaHSO₃ solution. One day after spraying, segments of equal area were cut from the control and treated leaves at corresponding positions and then placed in boiling water for 10 min. The ATP content in the solutions were measured using the luciferin-luciferase assay. The medium for measurement contained 50 mmol/L glycylglycine (pH 7.6), 10 mmol/L MgSO₄, 1 mmol/L EDTA, and 3 mg/mL luciferin. Experiments were repeated five times.

1.4 Measurement of chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters and electron transport rates (ETR) were measured with a pulse amplitude modulation (PAM) chlorophyll fluorometer (Walz, Effeltrich, Germany) according to the methods of Schreiber (1986) and van Kooten and Snel (1990). Before measurement, the sample was kept in the dark for 15 min. Then, modulated measuring light (1.6 kHz, 660 nm) was turned on and, after the Fo signal had been recorded, actinic light (> 645 nm, 300 µmol·m⁻²·s⁻¹) was turned on for 1 min and the signal dynamics continued to be recorded after the actinic light had been turned off. The fluorescence kinetics parameters were calculated using the following formulae. Photochemical efficiency (PSII):

\[ \frac{F_v}{F_m} = \frac{(F_m - F_o)}{F_m} \]

where \( F_v \) is fluorescence variable and \( F_m \) is fluorescence maximum. Photochemical quenching was calculated as:

\[ qp = \frac{(F_m' - F_s)}{(F_m' - F_o)} \]

where \( F_s \) is the steady state fluorescence yield and \( F_m' \) is light adapted fluorescence maximum and non-photochemical quenching was calculated as:

\[ qN = \frac{(F_m - F_m')}{(F_m - F_o)} \]

Experiments were repeated five times.

2 Results

2.1 Effect of different concentrations of NaHSO₃ and ATP on the photosynthetic rate of rice leaves and leaf discs, respectively

After solutions of 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 mmol/L NaHSO₃ had been applied (Fig. 1), net CO₂ assimilation rates in treated leaves were all enhanced compared with control leaves on the following day. Among these, the highest net CO₂ assimilation rate was observed in leaves treated with 1.0–2.0 mmol/L NaHSO₃. Net CO₂ assimilation rates, for example, in leaves of WT rice Kitaake, PC transgenic rice, and PC+PK transgenic rice were 24.8, 28.2, and 28.5 µmol CO₂·m⁻²·s⁻¹, respectively, which reflected an increase of 25.3%, 34.9%, and 41.1%, respectively, after spraying with 1.5 mmol/L of NaHSO₃. The increasing ranges of the net CO₂ assimilation rates were higher in transgenic rice compared with WT rice Kitaake. Figure 1 also shows that net CO₂ assimilation rates in three genotypes of rice decreased with increasing concentrations of NaHSO₃ solution greater than 2.0 mmol/L. In addition, ATP was added to the reaction medium of leaf discs to give a final concentration of 1, 2, 4, 8, and 16 µmol/L. Figure 1 shows that the photosynthetic oxygen release rates of leaf discs increased with increasing ATP concentrations in the reaction medium. For example, when the ATP concentration in reaction medium was 4 µmol/L, the photosynthetic oxygen release rates of leaf discs from WT rice Kitaake, PC transgenic rice, and PC+PK transgenic rice were 25.3, 29.1, and 29.4 µmol O₂·m⁻²·s⁻¹, respectively, which was an increase of 27.8%, 39.2%, and 42.2% compared with control, respectively.

Experimental results in Table 1 show that the \( Pn \) of leaves treated with NaHSO₃ increased, whereas \( Ci \)
declined and there was no remarkable change in Gs. In addition, Ci decreased more in transgenic rice leaves treated with NaHSO₃ than in WT rice Kitaake. It was obvious that the enhancement of Pn could not attribute to a change in Gs but, rather, to an increase in the change of the photosynthetic activity of mesophyll cells. These results are consistent with those of Wang et al. (2000).

2.2 Effects of NaHSO₃ and PMS on the photosynthetic rates of rice leaves under different PFD

Leaves were sprayed with 1.5 mmol/L NaHSO₃, 0.2 µmol/L PMS (a cofactor for catalysing cyclic photophosphorylation), or a mixture of both, or leaf discs were submerged in a reaction medium containing 4 µmol/L ATP. Photosynthetic rates were then measured under PFDs of 200, 400, 800, 1200, and 1600 µmol·m⁻²·s⁻¹ at 25 °C. Means ± SD, n = 5. Means sharing same letter were not significantly different, P < 0.01.

### Table 1 Changes in net photosynthetic rate (Pn), stomatal conductance (Gs) and intercellular concentration of CO₂ (Ci) in leaves of wild type rice Kitaake (WT), PEPC transgenic (PC) and PEPC+PPDK transgenic (PC + PK) rice treated with 1.5 mmol/L NaHSO₃

<table>
<thead>
<tr>
<th>Rice genotypes</th>
<th>Pn (µmol CO₂·m⁻²·s⁻¹)</th>
<th>Gs (µmol H₂O·m⁻²·s⁻¹)</th>
<th>Ci (µmol CO₂/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (WT) Control</td>
<td>19.8 ± 1.9 (100.0)</td>
<td>351.5 ± 30.7 (100.0)</td>
<td>368.5 ± 26.1 (100.0)</td>
</tr>
<tr>
<td>Treatment</td>
<td>24.2 ± 2.7 (122.2)</td>
<td>362.1 ± 35.9 (103.0)</td>
<td>345.2 ± 36.8 (93.7)</td>
</tr>
<tr>
<td>PEPC transgenic rice (PC) Control</td>
<td>20.9 ± 1.9 (100.0)</td>
<td>369.9 ± 33.8 (100.0)</td>
<td>378.3 ± 35.8 (100.0)</td>
</tr>
<tr>
<td>Treatment</td>
<td>26.9 ± 2.4 (128.7)</td>
<td>358.3 ± 32.9 (96.9)</td>
<td>328.5 ± 33.0 (86.8)</td>
</tr>
<tr>
<td>PEPC+PPDK transgenic rice (PC+PK) Control</td>
<td>20.7 ± 2.3 (100.0)</td>
<td>360.3 ± 36.1 (100.0)</td>
<td>376.4 ± 32.6 (100.0)</td>
</tr>
<tr>
<td>Treatment</td>
<td>27.2 ± 2.9 (131.4)</td>
<td>356.2 ± 32.4 (98.9)</td>
<td>322.6 ± 31.1 (85.7)</td>
</tr>
</tbody>
</table>

The values in parentheses are the percentage of control. Measurement was carried out under a PFD of 1 000 µmol photons · m⁻²·s⁻¹ at 25 °C. Means ± SD, n = 5. Means sharing same letter were not significantly different, P < 0.01.
for photophosphorylation). As a result, there was no additional effect of spraying leaves with the combination of NaHSO₃ and PMS. The light intensity-photosynthetic curve exhibited the following features: the photosynthetic rates of leaves (leaf discs) treated with NaHSO₃, PMS, ATP, or a mixture of NaHSO₃ and PMS were the same as that of the control under lower PFD, such as 200 µmol·m⁻²·s⁻¹, but were obviously higher

![Figure 2](image_url)

**Fig. 2.** Changes in photosynthetic rates in leaves and leaf discs of wild-type rice Kitaake (WT), phosphoenolpyruvate carboxylase (PEPC) transgenic rice (PC), and PEPC+phosphate dikinase (PPDK) transgenic (PC+PK) rice treated with NaHSO₃, PMS, and ATP under different PFD.

**Table 2** Effect of NaHSO₃, PMS and ATP on net photosynthetic rates of the leaves and leaf discs of wild type rice Kitaake (WT), PEPC transgenic (PC) and PEPC+PPDK transgenic (PC+PK) rice, respectively

<table>
<thead>
<tr>
<th></th>
<th>PFD (µmol·m⁻²·s⁻¹)</th>
<th>Control</th>
<th>+ NaHSO₃</th>
<th>+ PMS</th>
<th>PMS + NaHSO₃</th>
<th>+ ATP†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitaake (WT)</td>
<td>200</td>
<td>5.97 ± 0.3(100)</td>
<td>6.25 ± 0.4(104.6)</td>
<td>6.24 ± 0.4(104.5)</td>
<td>6.23 ± 0.3(104.4)</td>
<td>6.13 ± 0.5(102.7)</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>21.3 ± 1.3(100)</td>
<td>25.2 ± 2.2(118.3)</td>
<td>25.9 ± 1.8(121.6)</td>
<td>25.2 ± 2.1(118.3)</td>
<td>25.2 ± 1.5(118.3)</td>
</tr>
<tr>
<td>PEPC transgenic</td>
<td>200</td>
<td>7.03 ± 0.3(100)</td>
<td>7.38 ± 0.2(105.0)</td>
<td>7.33 ± 0.4(104.3)</td>
<td>7.29 ± 0.5(103.7)</td>
<td>7.22 ± 0.4(102.3)</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>23.1 ± 2.1(100)</td>
<td>29.8 ± 2.6(129.0)</td>
<td>29.5 ± 2.2(127.7)</td>
<td>29.6 ± 2.4(128.1)</td>
<td>29.9 ± 1.6(129.4)</td>
</tr>
<tr>
<td>Kitaake (PC)</td>
<td>200</td>
<td>7.12 ± 0.4(100)</td>
<td>7.35 ± 0.3(103.6)</td>
<td>7.39 ± 0.2(103.8)</td>
<td>7.33 ± 0.4(102.9)</td>
<td>7.19 ± 0.3(100.9)</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>22.9 ± 2.1(100)</td>
<td>30.3 ± 2.8(132.3)</td>
<td>29.3 ± 2.5(127.9)</td>
<td>29.4 ± 2.4(128.4)</td>
<td>30.3 ± 1.9(132.3)</td>
</tr>
</tbody>
</table>

†, µmol O₂·m⁻²·s⁻¹. Leaves were sprayed with 1.5 mmol/L NaHSO₃, or 0.2 µmol/L PMS or a mixture of both, respectively. Leaf discs were submerged in reaction medium containing 4 µmol/L ATP. The values in parentheses are the percentage. Measurement was carried out at 25 °C. Means ± SD, n = 5, significantly different, P < 0.01.
than that of control under higher PFD, such as over 800 µmol·m$^{-2}·$s$^{-1}$. Such differences become more obvious with increases in PFD. The fact that with the various treatment conditions the extent of the increase in the photosynthetic rate in transgenic rice was obviously greater than that in WT rice Kitaake, indicates that energy, as a limiting factor for photosynthesis, exhibited more extrusive under strong light. Therefore, ATP content was measured. Table 3 shows that the ATP content in leaves treated with 1.5 mmol/L NaHSO$_3$, 0.2 µmol/L PMS, or a mixture of both was increased obviously, by approximately 25%–28%, compared with control. However, the degree of the increase in ATP content was not significantly different between WT and transgenic rice.

2.3 Effect of NaHSO$_3$ on chlorophyll fluorescence parameters and the ETR of leaves in rice

Chlorophyll fluorescence features and the ETR of rice leaves also changed after treatment with NaHSO$_3$. Table 4 shows that, under control conditions, the PSII photochemical efficiency ($Fv$/$Fm$) and ETR were the highest in the early morning (Beijing time, 09:00 am), lowest in the middle of the day (Beijing time, 13:00 pm), and then started to recover slowly. However, under treatment conditions (after treatment NaHSO$_3$), the $Fv$/$Fm$ values of rice leaves did not change obviously in the early morning (Beijing time, 09:00 h), but, compared with control, increased significantly ($P<0.01$) by 6.9%, 11.2%, and 12.3% in WT rice Kitaake, PC transgenic rice, and PC+PK transgenic rice, respectively, in the middle of the day (Beijing time, 13:00 h). In addition, the ETR changed obviously after treatment with NaHSO$_3$. For example, compared with control, ETR increased significantly ($P<0.01$) by 6.8%, 31.3%, and 50.0% in the early morning, and by 12.3%, 41.3%, and 63.1% in the middle of the day in WT rice Kitaake, PC transgenic rice, and PC+PK transgenic rice, respectively. Statistical calculation showed that the values of $Fv$/$Fm$ and ETR increased more in transgenic rice compared with WT rice after treatment with NaHSO$_3$ ($P < 0.01$).

3 Discussion

The facts that the ATP content in rice leaves treated with NaHSO$_3$ at lower concentrations increased under light and that there was no additional effect of spraying leaves with a combination of NaHSO$_3$ and PMS (a co-factor for catalysing cyclic photophosphorylation) indicates that the enhancing effect of these compounds on the photosynthetic rate may be via the same mechanism (i.e. by increasing the supply of ATP for cyclic photophosphorylation; Wang et al. 2000, 2003; Wang and Shen 2002). Cyclic photophosphorylation could play an important role in alleviating photoinhibitory damage and enhancing limited C$_4$ photosynthesis.

3.1 Leaves treated with NaHSO$_3$ at lower concentrations alleviated photoinhibitory damage by increasing cyclic electron transport

In the present study, the light intensity-photosynthetic curve indicated that the photosynthetic rates of leaves (leaf discs) treated with NaHSO$_3$, PMS, ATP, or a mixture of NaHSO$_3$ and PMS were the same as that of control under lower PFD, such as 200 µmol·m$^{-2}·$s$^{-1}$, but were obviously higher than that of control under lower PFD, such as 800 µmol·m$^{-2}·$s$^{-1}$. Such differences become more obvious with increases in PFD. The fact that with the various treatment conditions the extent of the increase in the photosynthetic rate in transgenic rice was obviously greater than that in WT rice Kitaake, indicates that energy, as a limiting factor for photosynthesis, exhibited more extrusive under strong light. Therefore, ATP content was measured. Table 3 shows that the ATP content in leaves treated with 1.5 mmol/L NaHSO$_3$, 0.2 µmol/L PMS, or a mixture of both was increased obviously, by approximately 25%–28%, compared with control. However, the degree of the increase in ATP content was not significantly different between WT and transgenic rice.

<table>
<thead>
<tr>
<th></th>
<th>ATP contents (µmol/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control + NaHSO$_3$ + PMS</td>
</tr>
<tr>
<td>Kitaake (WT)</td>
<td>2.011 ± 0.112 (100)</td>
</tr>
<tr>
<td>PEPC transgenic</td>
<td>2.126 ± 0.131 (100)</td>
</tr>
<tr>
<td>Kitaake (PC)</td>
<td>2.117 ± 0.122 (100)</td>
</tr>
</tbody>
</table>

The values in parentheses are the percentage of control. Means ± SD, n = 5. Means sharing the same letter were not significantly different, $P < 0.01$. 

The facts that the ATP content in rice leaves treated with NaHSO$_3$ at lower concentrations increased under light and that there was no additional effect of spraying leaves with a combination of NaHSO$_3$ and PMS (a co-factor for catalysing cyclic photophosphorylation) indicates that the enhancing effect of these compounds on the photosynthetic rate may be via the same mechanism (i.e. by increasing the supply of ATP for cyclic photophosphorylation; Wang et al. 2000, 2003; Wang and Shen 2002). Cyclic photophosphorylation could play an important role in alleviating photoinhibitory damage and enhancing limited C$_4$ photosynthesis.

3.1 Leaves treated with NaHSO$_3$ at lower concentrations alleviated photoinhibitory damage by increasing cyclic electron transport

In the present study, the light intensity-photosynthetic curve indicated that the photosynthetic rates of leaves (leaf discs) treated with NaHSO$_3$, PMS, ATP, or a mixture of NaHSO$_3$ and PMS were the same as that of control under lower PFD, such as 200 µmol·m$^{-2}·$s$^{-1}$, but were obviously higher than that of control under lower PFD, such as 800 µmol·m$^{-2}·$s$^{-1}$.
higher PFD, such as over 800 μmol·m⁻²·s⁻¹. Such differences became more obvious with increasing in PFD (Fig. 2). The results indicated that more photoinhibition of photosynthesis occurred in control leaves (sprayed with distilled water) than in NaHSO₃-treated leaves because NaHSO₃ at low concentrations can promote cyclic electron transport (Wang et al. 2000). Cyclic electron transport around PSI has been shown to provide extra ATP for different cellular processes (e.g. adaptation to stress conditions). During oxygenic photosynthesis, PSII and PSI cooperate to achieve a linear electron flow from H₂O to NADP⁺ and to generate a transmembrane proton gradient driving ATP synthesis. However, ATP can also be produced by the sole PSI through cyclic electron transfer reactions (Arnon 1959). For example, during strong light treatments, PSI is more stable than PSII and because PSI photoinhibition has seldom been observed in vivo (Havaux and Eyletters 1991), the major site of photoinhibition has been assumed to be PSII. When PSII is inhibited, cyclic photophosphorylation does not require PSII, but it generates ATP and is therefore a likely source of energy for the repair of photodamage to PSII (Joët et al. 2002). Cyclic electron transport is the light-mediated transfer of electrons from the plastoquinone pool, via PSI and ferredoxin, back to the plastoquinone pool (Bendall and Manasse 1995). In Cyanobacteria, cyclic electron flow around PSI has been shown to provide extra ATP for different cellular processes (e.g. adaptation to salt stress conditions; Jeanjean et al. 1993). In C₃ plants, cucumber (Cucumis sativus L.) plants grown at low PFD (120–150 μmol·m⁻²·s⁻¹) were photoinhibited by a 3 h exposure in air to 10-fold the light intensity experienced during growth. It was found that PSII electron transport and non-cyclic photophosphorylation were inhibited by approximately 50%, whereas cyclic photophosphorylation was less inhibited and PSI electron transport and light-induced proton uptake were unaffected. The results confirm that, following photoinhibition treatment of higher plant leaves, electron transport associated with PSII is significantly decreased, whereas PSI-related electron transport remains unaffected (Critchley 1981). Cyclic electron transport is a process that has been suggested to play an important role in protecting against photo-oxidative stress (Heber and Walker 1992). This mechanism enables the generation of a proton gradient across the thylakoid membrane without NADP⁺ reduction by rerouting electrons of reduced PSI acceptors towards the intersystem carriers. Cyclic electron transport may also reduce the photoinhibitory effect of pseudocyclic electron transport (i.e. the photoreduction of oxygen) via the Mehler reaction, by rerouting linear electron transport. Furthermore, acidification of the thylakoid lumen by proton translocation during cyclic electron transport can increase non-photochemical quenching by different mechanisms and, thus,
prevent over-reduction of the electron carriers and subsequent photoinactivation of the electron transport chain (Teicher et al. 2000).

### 3.2 Leaves treated with NaHSO₃ at lower concentrations enhance limited C₄ photosynthesis by supplying extra ATP

The C₄ photosynthesis has a number of features of CO₂-concentrating mechanisms in general. These components have been defined earlier by Badger and Spalding (2000) as follows:

1. An active, photosynthetically driven CO₂-capture system.
2. A supply of photosynthetic energy. In C₄ photosynthesis, the reaction, catalysed by PPDK, consumes, in addition, two ATP per CO₂ assimilated. One ATP is required directly by PPDK, the second ATP is needed for the conversion of the reaction product AMP into ADP, catalysed by adenylate kinase. Thus, the C₄ cycle is an ATP-driven CO₂ pump, using two ATP per CO₂ transferred.
3. An intermediate pool of captured CO₂. These pools consist of C₄ acids, such as malate and aspartate.
4. A mechanism to release CO₂ from the intermediate pool.
5. A compartment in which to concentrate CO₂ around Rubisco.
6. A means to reduce leakage of CO₂ from sites of elevated CO₂.
7. Modification of the kinetic properties of Rubisco.

In short, the simplest theoretical single-celled CO₂-concentrating system that could be engineered into a C₃ leaf would be PEP carboxylation in the cytosol, oxaloacetate transport into the chloroplast, decarboxylation of oxaloacetate to PEP by PEP carboxykinase in the chloroplast, followed by transport of PEP back to the cytosol. This could be achieved at the expense of ATP for CO₂ transferred. Therefore, the ATP supply may become the limiting factor to concentrate CO₂ in rice leaves transformed with an exogenous PEPC gene and exogenous PEPC+PPDK genes. That is, cyclic photophosphorylation enhanced by NaHSO₃ at lower concentrations provides extra ATP for C₄ photosynthesis.

As a result, photosynthetic rates increased obviously in all rice genotypes, but the increase was greater in transgenic rice after treatment with NaHCO₃.

A further problem worthy of consideration is that the increased ATP content is not different among different rice genotypes after treatment with NaHSO₃; however, the photosynthetic rate and photoinhibition-tolerance capacity in transgenic rice increased more than in untransformed WT rice Kitaake under strong light. There were many factors impacting on the photoinhibition-tolerance capacity of rice, such as short ATP supply etc. In addition, the coordinated expression of the exogenous C₄ gene in C₃ cells also plays an important role. Experimental evidence indicates that the introduction and expression of an exogenous C₄ gene enhances the existing, and limited, C₄ photosynthetic cycle in C₃ cells. The result of the present study are also consistent with those of a previous report (Ji et al. 2004).

### References


(Managing editor: Ping HE)