Running title:
Chloroplast ATP synthase and Cytochrome b6/f complexes

Corresponding author: Wataru Yamori
Department of Applied Plant Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai, 981-8555, Japan.
Tel: +81-22-717-8767; Fax: +81-22-717-8765
E-mail: wataru.yamori@biochem.tohoku.ac.jp

Research Category
Bioenergetics and Photosynthesis
The roles of ATP synthase and the cytochrome b6/f complexes in limiting chloroplast electron transport and determining photosynthetic capacity

Authors
Wataru Yamori1,2, Shunichi Takahashi1, Amane Makino2, G. Dean Price1, Murray R. Badger1 and Susanne von Caemmerer1

1Molecular Plant Physiology Cluster, Plant Science Division, Research School of Biology, The Australian National University, Canberra, ACT, 2601, Australia
2Department of Applied Plant Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai, 981-8555 Japan

Manuscript information: 6 figures and 3 supplemental figures
Footnotes

Financial source

This work was supported by a grant from the Japan Society for the Promotion of Science Postdoctoral Fellowships for Research Abroad (to W.Y.).
Abstract

In C₃ plants, CO₂ assimilation is limited by RuBP regeneration rate at high CO₂. RuBP regeneration rate in turn is determined by either the chloroplast electron transport capacity to generate NADPH and ATP or the activity of Calvin cycle enzymes involved in regeneration of RuBP. Here, transgenic tobacco (Nicotiana tabacum L. cv. W38) expressing an antisense gene directed at the transcript of either the Rieske FeS protein of the cytochrome b₆/f complex or the δ subunit of chloroplast ATP synthase have been used to investigate the effect of a reduction of these complexes on chloroplast electron transport rate. Reductions in δ subunit of ATP synthase content did not alter chlorophyll, cytochrome b₆/f complex or Rubisco content, but reduced electron transport rates estimated either from measurements of chlorophyll fluorescence or CO₂ assimilation rates at high CO₂. Plants with low ATP synthase content exhibited higher NPQ and achieved higher electron transport rates per ATP synthase than wild type. The proportional increase in electron transport rates per ATP synthase complex was greatest at 35°C, showing that the ATP synthase activity can vary in vivo. In comparison, there was no difference in the electron transport rate per cytochrome b₆/f complex in plants with reduced b₆/f content and wild type. The electron transport rates decreased more drastically with reductions in cytochrome b₆/f complex than ATP synthase content. This suggests that chloroplast electron transport rate is more limited by cytochrome b₆/f than ATP synthase content and is a potential target for enhancing photosynthetic capacity in crops.
Introduction

Plants capture light energy with their light-harvesting systems, including chlorophylls and carotenoids, and drive photosynthetic electron transport through the thylakoid membranes of the chloroplasts. Electrons excised from water in photosystem II (PSII) are ultimately transferred to NADP⁺ via photosystem I (PSI), resulting in production of NADPH. This process is known as linear electron transport. At the same time, this linear electron transport which passes through the cytochrome (Cyt) b₆/f complex generates a proton gradient across the thylakoid membrane (ΔpH) (Allen, 2003). Together with the proton gradient generated by the water-splitting complex associated with PSII, these proton gradients enable ATP production by the ATP synthase complex and help to regulate non-photochemical quenching of excitation energy (Muller et al., 2001). There is also a cyclic electron transport which depends on the PSI photochemical reactions and also passes through the Cyt b₆/f complex. The cyclic electron transport can generate a ΔpH and drives ATP synthesis by ATP synthase without concomitant generation of NADPH (Shikanai, 2007).

ATP and NADPH generated by light reactions are utilized primarily in the Calvin cycle and photorespiratory cycle. The activity and regulation of the Cyt b₆/f complex and the ATP synthase are thus key components determining the rate of NADPH and ATP production for CO₂ fixation. Photosynthetic CO₂ assimilation rate can be viewed as being limited either by the capacity of Rubisco to consume RuBP (at lower CO₂) or by the capacity of the chloroplast electron transport to generate ATP and NADPH for RuBP regeneration (at higher CO₂) (Farquhar et al., 1980). However, within this framework of limitations, significant uncertainties remain in our understanding of how electron transport and ATP synthesis are coordinated and affect electron transport capacity and photosynthesis (Baker et al., 2007).

Previous work has shown that a targeted reduction in Cyt b₆/f complex content caused reductions in the chloroplast electron transport rate and CO₂ assimilation rate at 25°C (Price et al., 1995, 1998; Ruuska et al., 2000). Therefore, the electron flow through Cyt b₆/f complex is considered to be a key rate-limiting steps for RuBP regeneration at 25°C. However, there are few studies that have considered the role of chloroplast ATP synthase as a limiting factor for the thylakoid reactions. Recent studies have documented that the in vivo activity of ATP synthase is modulated, especially at low or high CO₂ concentration where CO₂ assimilation is restricted either by CO₂ concentration or end-product limitation (Kanazawa & Kramer, 2002; Kramer et al., 2004; Baker et al., 2007). The conductivity of proton efflux from the lumen (gH⁺) through the ATP synthase could be modulated to regulate the thylakoid proton motive force (pmf) (Kramer et al., 2004), providing flexibility in the ratio of ATP production per H⁺.

We used transgenic tobacco (Nicotiana tabacum L. cv. W38) plants expressing an antisense gene directed at the transcript of either the Rieske FeS protein of the Cyt b₆/f complex or the δ subunit of chloroplast ATP synthase (Price et al., 1995) to investigate the effect that a reduction of these complexes has on chloroplast electron transport rate and CO₂ assimilation rate. Combined measurements of gas exchange and chlorophyll fluorescence were made over a range of CO₂ concentrations and leaf temperatures. We show that at high CO₂ when the rate of RuBP regeneration limits CO₂ assimilation, chloroplast electron transport rate is more limited by Cyt b₆/f than ATP synthase content and confirm that ATP synthase activity is modulated in vivo. We suggest that increasing Cyt b₆/f content may be a useful
biomolecular target for enhancing leaf photosynthesis for improved crop yield (von Caemmerer & Evans, 2010).

Results

CO₂ assimilation rate and physiological components of photosynthesis

CO₂ assimilation rate at 380 μmol mol⁻¹ CO₂ at high light (A₃₈₀) was strongly decreased with reductions in the content of either the δ subunit of ATP synthase complex or the Rieske FeS subunit of the Cyt b₆/f complex (Fig. 1). However, the comparative extent of the reductions of A₃₈₀ was greater in anti-Rieske FeS plants than in anti-ATP synthase (δ) plants.

The contents of several photosynthetic components, including Rubisco and chlorophyll, were similar among wild type, anti-Rieske FeS plants and anti-ATP synthase (δ) plants (Fig. 2). Chl a/b ratio in WT was 3.14 ± 0.06 and similar to anti-Rieske FeS plants (3.06 ± 0.15) and anti-ATP synthase (δ) plants (3.09 ± 0.15). It was previously shown that reduction in the ATP synthase (δ) subunit led to a reduction in ATP synthase (Price et al., 1995) and that the reduction on Rieske FeS protein led to reduction in the complete Cyt b₆/f complex (Price et al., 1998). We therefore assume that alterations in photosynthetic properties are primarily the result of the reduction in either ATP synthase or Cyt b₆/f complex.

Chlorophyll fluorescence

The electron transport rate (ETR) was greater at 40°C than at 25°C in WT. The parameter of 1-qL, which reflects the plastoquinone reduction state (Kramer et al., 2004; Baker et al., 2007), was lower at 40°C than at 25°C. NPQ was lower at 40°C than at 25°C (Fig. 3).

Reduction in Rieske FeS contents greatly reduced ETR at 25°C and 40°C. These resulted in a concomitant over-reduction of the plastoquinone pool (high 1-qL) and a low transthylakoid pH gradient (ΔpH) (low NPQ), because of a reduced ability to transport protons across the thylakoid membrane. The reduction state of PSII centers could not be associated with a build-up of transthylakoid ΔpH, since NPQ was lower in anti-Rieske FeS plants than wild type.

Reduction in ATP synthase (δ) contents increased NPQ, suggesting an increase transthylakoid ΔpH due to a reduction in the flux of protons outwards through the ATP synthase. The limitation imposed on CO₂ fixation by a reduction in ATP supply led to the reduction of potential for ETR, causing an increase in the reduction state of the plastoquinone pool (high 1-qL). The reduction state of PSII centers was associated with a build-up of transthylakoid ΔpH, since NPQ was greater in anti-ATP synthase (δ) plants than wild type.

Activation state of NADP malate dehydrogenase (NADP-MDH)

Activation state of NADP-MDH, which is an indicator of NADPH/NADP⁺ ratio, decreased by 7–8% at 40°C compared to 25°C in WT (Fig. 4).

The anti-Rieske FeS plants had an impaired ETR and a low NPQ that restricts NADPH and ATP...
synthesis. The activation state of NADP-MDH strongly decreased in anti-Rieske FeS plants. In contrast, anti-ATP synthase (δ) plants had an impaired ETR and a high NPQ and the activation state of NADP-MDH was also strongly decreased.

NADP-MDH activity is correlated with the redox status of the stroma and NADPH availability (Scheibe & Stitt 1988). Reductions in ETR via reductions in Rieske FeS protein caused decreases in 1-qL and NADP-MDH activation (Figs 3 & 4). Therefore, in anti-Rieske FeS plants, an oxidation of the stroma could inhibit many of the redox-sensitive enzymes within the stroma, including several in the Calvin cycle. We also observed decreases in 1-qL and NADP-MDH activation at high temperature (Figs 3 & 4), implying that the heat-induced decline in photosynthesis would be also affected by, at least, the redox status of the stroma (Schrader et al., 2004; Sharkey, 2005).

RuBP regeneration and chloroplast electron transport capacity (Jg)

CO₂ assimilation rate (A) versus intercellular CO₂ concentration (Ci) was measured to determine RuBP regeneration and/or electron transport limited CO₂ assimilation rate at high CO₂, and these were used to calculate actual electron transport rates (Jg) as described in the Material and Methods (Figs S1 and S2). The A-Ci curves showed a transition from Rubisco limited-A at lower CO₂ concentrations to RuBP regeneration limited-A at higher CO₂ concentrations at all leaf temperatures in all plants except those plants with the lowest ATP synthase (δ) or Rieske FeS content where CO₂ assimilation was always electron transport limited.

Reductions in contents either of ATP synthase (δ) or Rieske FeS led to a decrease in Jg at 25°C (Fig. 5A, B). In anti-Rieske FeS plants, Jg per Rieske FeS content was constant irrespective of Rieske FeS content (Fig. 5D). However, in anti-ATP synthase (δ) plants, the Jg per ATP synthase (δ) content increased with reductions in ATP synthase (δ) content (Fig. 5C), indicating that in vivo ATP synthase activity of an individual ATP synthase complex was enhanced in anti-ATP synthase (δ) plants.

Temperature responses of Jg in anti-ATP synthase (δ) plants and anti-Rieske FeS plants are shown in Figure 6. We found that the temperature dependence of Jg /Rieske FeS content was the same for wild type and plants with a range of Rieske FeS content. However, the temperature dependence of Jg /ATP synthase (δ) content varied with the ATP synthase levels of the plants being measured, being greatest at high temperature in transgenic plants with low ATP synthase content.

Discussion

The data presented here show that there is a strong control of chloroplast electron transport and photosynthetic capacity by the level and activity of both the Cyt b₆/f and ATP synthase complexes. However, the manner in which each complex does this and their relative contributions is distinctly different. It was clearly evident that the Cyt b₆/f complex exhibited much tighter control of electron transport capacity and photosynthesis than that of the ATP synthase complex (Figs 1 & 5). A significant basis for this appears to lie in the fact that there is a strong potential for an individual ATP synthase complex to modulate its
proton conductance and ATP synthesis per electron transport, while the Cyt \( b_6/f \) complex has much less flexibility (Figs 5C & 6C).

**ATP synthase activity varies in vivo**

When the ATP synthase complex content was reduced, the evidence clearly indicates that the actual chloroplast electron transport rate per ATP synthase complex increased and this was greatest at high temperature (Fig. 5). However, the increased rate of ATP synthase does not fully compensate for the reduced amount of ATP synthase, but ATP synthase activity goes faster when there is less of it. This supports the notion that the activity of an ATP synthase complex can vary *in vivo* when ATP synthase content is reduced. This change in activity could be due to changes in substrate availability (stromal ADP, Pi and trans-thylakoid proton motive force (pmf)), the activation state of the complex or the proton stoichiometry per ATP.

There have been several reports about the nature of the modulation of ATP synthase activity. For example, the ATP synthase is regulated by \( \text{pmf} \) and by reduction of \( \gamma \)-subunit thiols via thioredoxin (Kramer & Crofts, 1989; Ort & Oxborough, 1992; Fischer *et al*., 2000; McCarty, 2005; McCalluma & McCarty, 2007). We found that reductions in ATP synthase content increased NPQ and probably the transthylakoid \( \Delta \text{pH} \) as previously reported (Price *et al*., 1995). It has also been suggested that ATP synthase senses the status of stromal metabolites either directly or indirectly (Kramer *et al*., 2004) and it has been suggested that its activity can be modulated by altering Pi levels (Kanazawa & Kramer, 2002; Takizawa *et al*., 2008). A number of Calvin cycle enzyme activities are regulated by the chloroplast ferredoxin-thioredoxin pathway (e.g., Rubisco via Rubisco activase in Fig. S3, GA3PDH, SBPase, FBPase, PRK). The reduction in NADP-MDH activation state with reduction in ATP synthase content suggests that the activity of these redox regulated enzymes of the Calvin cycle may also have decreased. This would decrease ATP consumption such that changes in ADP and Pi levels may not be as large as otherwise expected similar to what was observed for plants with reduced Cytochrome \( b_6/f \) complex (Ruuska *et al*., 2000). Nevertheless, both substrate availability and/or the activation state of the ATP synthase complex via thioredoxin system could be enhanced in plants with low ATP synthase contents.

Recent estimations of proton stoichiometry indicated that the \( \text{H}^+/\text{ATP} \) ratio is 4.66 (Baker *et al*., 2007). Interestingly, there have been reports that the proton stoichiometry in ATPase may vary depending on environmental conditions in *Escherichia coli* (Schemidt *et al*., 1995, 1998). Thus, it may also be possible that the proton stoichiometry in ATP synthase varied between WT and anti-ATP synthase line, since their physiological states (e.g., transthylakoid \( \Delta \text{pH} \)) was different.

**Cytochrome \( b_6/f \) content is rate limiting for chloroplast electron transport**

The Cyt \( b_6/f \) complex has a unique role in chloroplast electron transport, as it can act in both linear electron transport (production of ATP and NADPH) and cyclic electron transport (ATP generation only). There was a strong linear relationship between chloroplast electron transport rate and Cyt \( b_6/f \) content such that electron transport per Cyt \( b_6/f \) content was the same for plants with a large range of Rieske FeS content.
(Figs 1 & 5) similar to previous observations (Price et al., 1995, 1998).

The photosynthetic model of Farquhar et al. (1980) suggests that CO₂ assimilation in C₃ plants is limited by the rate of RuBP regeneration at high CO₂ and that RuBP regeneration rate in turn is determined by either the chloroplast electron transport capacity to generate NADPH and ATP or the activity of Calvin cycle enzymes involved in regeneration of RuBP. There have been a number of studies using transgenic plants to investigate whether Calvin enzymes limit the rate of RuBP regeneration and only sedoheptulose-1,7-bisphosphatase (SBPase) has been suggested as a possible candidate for a rate limiting step (for a review, see Raines, 2003, 2006).

This is the first time that the dependence of electron transport rate on Cyt b₆/f content and ATP synthase content have been compared. Our results can be interpreted to suggest that measurements of CO₂ assimilation rate at high CO₂ can be used to infer Cyt b₆/f content of leaves (see also Yamori et al., 2010a; Niinemets & Tenhunen, 1997). The assumption that RuBP regeneration rate is limited by chloroplast electron transport rate and Cyt b₆/f content rather than ATPase content may provide a robust mechanism for scaling carbon uptake from leaf photosynthesis to canopies, and ecosystems. This approach would be complementary to the common practice of using the initial slope of the CO₂ response curve to quantify the Rubisco content of leaves (von Caemmerer & Farquhar, 1981; Long & Bernacchi, 2003; Yamori et al., 2006a, 2010a).

Enhancing C₃ photosynthesis

It has been argued that a new “green revolution” is needed in world agriculture to increase crop yields for food security (Fischer & Edmeades, 2010). Increasing leaf photosynthetic capacity provides one attractive avenue to drive increases in crop yields (see, Long et al., 2006; Peterhansel et al., 2008). In a future high CO₂ world, C₃ photosynthesis will be increasingly limited by RuBP regeneration. The observation that the introduction of a parallel electron carrier between cytochrome f and photosystem I through the expression of Porphyra cytochrome c₆ in Arabidopsis conferred more rapid electron flow in vitro and enhanced plant growth (Chida et al., 2007), supports the notion of the strong control that intersystem electron transport through the Cyt b₆/f complex has on photosynthetic capacity. Research is needed to explore how the levels of Cyt b₆/f and ATP synthase complexes are regulated in the thylakoid membrane and what strategies may be employed to increase their content. This will be challenging given that both complexes contain both nuclear and chloroplast encoded subunits and that there appears to be strong post-transcriptional control of complex synthesis and assembly in the chloroplast (Leister, 2003).

Materials & Methods

Plant materials and growth conditions

Nicotiana tabacum L. cv Wisconsin 38 plants (W38) and the progeny of several transformants of anti-Rieske FeS tobacco and anti-ATP synthase tobacco which have reduced amounts of the chloroplast Cyt b₆/f and ATP synthase were grown in controlled environmental growth cabinets (Price et al., 1995).
Plants were grown at irradiance of 60 ~ 80 µmol m\(^{-2}\) s\(^{-1}\) with a photoperiod of 20h and ambient CO\(_2\) concentration. The day/night air temperatures were 30/25°C, and the relative humidity was 70%. Plants were grown in 5 L pots in garden mix containing approximately 2 g L\(^{-1}\) of a slow-release fertilizer (Osmocote, Scotts Australia, Castle Hill, Australia) and watered daily. The low irradiance was selected to minimize the difference in the growth rate of plants and the capacity of CO\(_2\) assimilation at the growth condition and to minimize the differences in the growth rate of plants and the capacity of CO\(_2\) assimilation at the growth condition (Ruuska et al., 2000).

**Gas exchange and fluorescence measurements**

CO\(_2\) gas exchange of leaves was measured with a portable gas exchange system (LI-6400, Li-COR, Lincoln, NE, USA). The whole portable gas exchange system was enclosed in a temperature-controlled cabinet (Yamori et al., 2005, 2006b, 2008, 2009, 2010b). The CO\(_2\) assimilation rate (\(A\)) versus intercellular CO\(_2\) concentration (\(C_i\)) was measured at a light intensity of 1200 µmol photons m\(^{-2}\) s\(^{-1}\) under several measurement temperatures. \(A\)-\(C_i\) curves were fitted with the C\(_3\) photosynthesis model (Farquhar et al., 1980), using the Rubisco kinetic constants and temperature dependencies in tobacco (Bernacchi et al., 2001). CO\(_2\) assimilation rates at high CO\(_2\) and measured rates of dark respiration (\(R_d\)) were used to calculate actual rates of chloroplast electron transport required to satisfy NADPH consumption (\(J_g\) (µmol m\(^{-2}\) s\(^{-1}\))):

\[
J_g = \frac{(A + R_d)(4 + 8\Gamma^*)}{C_i - \Gamma^*}
\]

where \(C_i\) (µmol mol\(^{-1}\)) is intercellular CO\(_2\), \(\Gamma^*\) (µmol mol\(^{-1}\)) is the CO\(_2\) compensation point in the absence of day respiration (von Caemmerer & Farquhar 1981).

Chlorophyll \(a\) fluorescence was also determined by an integrated fluorescence chamber head (LI-6400, LI-6400-40 leaf chamber fluorometer, LI-COR). The quantum yield of photosystem II [\(\Phi_{PSII} = (F_{m}' - F')/(F_m')\)], photochemical quenching [\(qP = (F_m' - F')/(F_m' - F_o')\)], non-photochemical quenching [NPQ = \((F_m - F_m')/F_m')\], and the fraction of PSII centers in the open state (with QA oxidized) [\(qL = qP x (F_o' / F')\)] were calculated (Baker et al., 2007, 2008). The electron transport rate (ETR) was calculated as ETR = 0.5 x absI x \(\Phi_{PSII}\), where 0.5 is the fraction of absorbed light reaching PS II and absI is absorbed irradiance taken as 0.85 of incident irradiance (Genty et al., 1989).

**Determination of Rieske FeS of Cytochrome b\(_{6}/f\) complex, \(\delta\) subunit of ATP synthase, Rubisco activase and chlorophyll**

Immediately after the measurements of gas exchange, leaf discs were taken and immersed in liquid nitrogen and stored at -80°C. The frozen leaf sample was ground in liquid nitrogen and homogenized in an extraction buffer (Yamori & von Caemmerer, 2009; Yamori et al., 2010a). The content of Rieske FeS of Cyt \(b_{6}/f\) complex and \(\delta\) subunit of ATP synthase was quantified by immunoblotting with anti-Rieske FeS antibody and anti-ATP synthase (\(\delta\)) antibody (Agrisera, Vännäs, Sweden). Rubisco activase was also quantified by immunoblotting with anti-activase antibody (Yamori & von Caemmerer, 2009). Chlorophyll...
was extracted in 80% (v/v) acetone and determined (Porra et al., 1989). The leaf extract of one wild type leaf was selected as a standard (100%) and included as a dilution series on gels. The protein content of other samples was referenced against this standard.

**Determinations of Rubisco catalytic sites, Rubisco activation state and NADP-MDH activation state**

Samples used for the Rubisco activation assay and chloroplast NADP-MDH assay were collected from a leaf equilibrated at steady-state conditions in the gas exchange chamber. After gas exchange had reached the steady-state rate for at least 30 minutes at a given leaf temperature, the leaf section in the chamber was taken out and immediately frozen in liquid nitrogen. Rubisco catalytic sites and Rubisco activation state were determined by the stoichiometric binding of 14C-carboxy-arabinol-P2, whereas chloroplast NADP-MDH activation state was assayed by monitoring NADH oxidation at 340 nm (Yamori & von Caemmerer, 2009).

**Statistical analysis**

Statistical comparison of the regressions shown in Figures 1A & B and Figures 5A & B were analyzed with a separate slopes model using the software package Statistica.

**Acknowledgements**

We would like to thank Dr. John Evans for his generous advice and Simon Dwyer for help with the statistical analysis.
Reference


McCarty RE (2005) ATP synthase of chloroplast thylakoid membranes: A more in depth characterization


Figure Legends

Figure 1
CO₂ assimilation rates at 380 μL L⁻¹ CO₂ concentration at 1200 μmol photons m⁻² s⁻¹ (A₃₈₀) at 25°C in antisense plants with a variety of δ subunit of chloroplast ATP synthase (A) and in antisense plants with a variety of Rieke FeS contents (B). The regression lines are shown in each figure; A) \( y = (0.046±0.003)x + (1.906±0.16) \), \( R^2 = 0.93 \); B) \( y = (0.067±0.003)x - (0.149±0.190) \), \( R^2 = 0.97 \). Statistical comparison of regressions showed them to be significantly different at \( P < 0.000001 \).

Figure 2
Content of Rubisco, Chlorophyll, Rieske FeS protein of the Cytochrome b₅f complex and δ subunit of chloroplast ATP synthase in antisense plants with a variety of δ subunit of chloroplast ATP synthase (A, C, E & G) and in antisense plants with a variety of Rieke FeS contents (B, D, F & H). Contents of Rieke FeS protein and δ subunit of chloroplast ATP synthase in antisense lines were shown as a percentage relative to WT.

Figure 3
Chlorophyll fluorescence parameters at 380 μL L⁻¹ CO₂ concentration at 1200 μmol photons m⁻² s⁻¹ at 25°C (open symbol) or 40°C (closed symbol) in antisense plants with a variety of δ subunit of chloroplast ATP synthase (A, C & E), and in antisense plants with a variety of Rieke FeS contents (circles) (B, D & F). A, B: Electron transport rate (ETR). C, D: \( 1-q_L \) which reflects the plastoquinone reduction state. E, F: Non-photochemical quenching (NPQ). The data in wild type is shown in triangles, whereas the data in antisense plants is shown in circles. The regression lines are shown in each figure; A) \( R^2 = 0.92 \) at 25°C, \( R^2 = 0.87 \) at 40°C; B) \( R^2 = 0.94 \) at 25°C, \( R^2 = 0.87 \) at 40°C; C) \( R^2 = 0.95 \) at 25°C, \( R^2 = 0.75 \) at 40°C; D) \( R^2 = 0.95 \) at 25°C, \( R^2 = 0.91 \) at 40°C; E) \( R^2 = 0.83 \) at 25°C, \( R^2 = 0.70 \) at 40°C; F) \( R^2 = 0.77 \) at 25°C, \( R^2 = 0.77 \) at 40°C.

Figure 4
NADP-MDH activation state at 380 μL L⁻¹ CO₂ concentration at 1200 μmol photons m⁻² s⁻¹ at 25°C (closed symbol) or 40°C (open symbol) in wild-type (triangles) and antisense plants with a variety of δ subunit of chloroplast ATP synthase (circles) (A), and wild-type (triangles) and antisense plants with a variety of Rieke FeS contents (circles) (B).

Figure 5
The capacity of RuBP regeneration (\( J_g \)) and the \( J_g \) per ATP synthase (δ) content at 25°C in antisense plants with a variety of δ subunit of chloroplast ATP synthase (A and C) and the capacity of RuBP regeneration (\( J_g \)) and the \( J_g \) per Rieke FeS content at 25°C in antisense plants with a variety of Rieke FeS content (B and D). \( J_g \) was calculated from measurements of CO₂ assimilation rate at high CO₂ as described in the Materials and Methods section. The regression lines are shown in each figure. Regression coefficient (\( R^2 \)); A) \( R^2 = \)
0.95; B) $R^2 = 0.98$; C) $R^2 = 0.80$; D) $R^2 = 0.17$. Statistical comparison of regressions shown in A) and B) showed them to be significantly different at $P < 0.00001$.

Figure 6
Temperature responses of chloroplast electron transport ($J_g$) and the $J_g$ per ATP synthase ($\delta$) content in antisense plants with a variety of $\delta$ subunit of chloroplast ATP synthase (A and C) and temperature responses of $J_g$ and the $J_g$ per Rieke FeS content in antisense plants with a variety of Rieke FeS content (B and D). Three groups were classified with respect to ATP synthase ($\delta$) content or Rieske FeS content: Rieske FeS: WT (approximately 100%), plants with intermediate Rieske FeS level (58–85%) and plants with low Rieske FeS level (27–29%); ATP synthase ($\delta$): WT (approximately 100%), plants with intermediate ATP synthase ($\delta$) level (48–75%) and plants with low ATP synthase ($\delta$) level (22–26%). Data represent means ±SE, n = 3–4.
Figure 1

CO₂ assimilation rates at 380 μL L⁻¹ CO₂ concentration at 1200 μmol photons m⁻² s⁻¹ (A₃₈₀) at 25°C in antisense plants with a variety of δ subunit of chloroplast ATP synthase (A) and in antisense plants with a variety of Rieske FeS contents (B). The regression lines are shown in each figure: A) y = (0.046±0.003)x + (1.906±0.16), R² = 0.93; B) y = (0.067±0.003)x – (0.149±0.190), R² = 0.97. Statistical comparison of regressions showed them to be significantly different at P < 0.000001.
Figure 2
Content of Rubisco, Chlorophyll, Rieske FeS protein of the Cytochrome $b_{6}/f$ complex and δ subunit of chloroplast ATP synthase in antisense plants with a variety of δ subunit of chloroplast ATP synthase (A, C, E & G) and in antisense plants with a variety of Rieske FeS contents (B, D, F & H). Contents of Rieske FeS protein and δ subunit of chloroplast ATP synthase in antisense lines were shown as a percentage relative to WT.
Figure 3

Chlorophyll fluorescence parameters at 380 μL L⁻¹ CO₂ concentration at 1200 μmol photons m⁻² s⁻¹ at 25°C (open symbol) or 40°C (closed symbol) in antisense plants with a variety of δ subunit of chloroplast ATP synthase (A, C & E), and in antisense plants with a variety of Rieke FeS contents (circles) (B, D & F). A, B: Electron transport rate (ETR). C, D: 1-qL which the plastoquinone reduction state. E, F: Non-photochemical quenching (NPQ). The data in wild type is shown in triangles, whereas the data in antisense plants is shown in circles. The regression lines are shown in each figure; A) $R^2 = 0.92$ at 25°C, $R^2 = 0.87$ at 40°C; B) $R^2 = 0.94$ at 25°C, $R^2 = 0.87$ at 40°C; C) $R^2 = 0.95$ at 25°C, $R^2 = 0.75$ at 40°C; D) $R^2 = 0.95$ at 25°C, $R^2 = 0.91$ at 40°C; E) $R^2 = 0.83$ at 25°C, $R^2 = 0.70$ at 40°C; F) $R^2 = 0.77$ at 25°C, $R^2 = 0.77$ at 40°C.
Figure 4

NADP-MDH activation state at 380 μL L⁻¹ CO₂ concentration at 1200 μmol photons m⁻² s⁻¹ at 25 °C (closed symbol) or 40 °C (open symbol) in wild-type (triangles) and antisense plants with a variety of δ subunit of chloroplast ATP synthase (circles) (A), and wild-type (triangles) and antisense plants with a variety of Rieke FeS contents (circles) (B).
Figure 5

The capacity of RuBP regeneration ($J_g$) and the $J_g$ per ATP synthase (δ) content at 25°C in antisense plants with a variety of δ subunit of chloroplast ATP synthase (A and C) and the capacity of RuBP regeneration ($J_g$) and the $J_g$ per Rieke FeS content at 25°C in antisense plants with a variety of Rieke FeS content (B and D). $J_g$ was calculated from measurements of CO$_2$ assimilation rate at high CO$_2$ as described in the Materials and Methods section. The regression lines are shown in each figure. Regression coefficient ($R^2$); A) $R^2 = 0.95$; B) $R^2 = 0.98$; C) $R^2 = 0.80$; D) $R^2 = 0.17$. Statistical comparison of regressions shown in A) and B) showed them to be significantly different at $P < 0.00001$. 

www.plantphysiol.org on January 22, 2018 - Published by www.plantphysiol.org
Copyright © 2010 American Society of Plant Biologists. All rights reserved.
Figure 6

Temperature responses of chloroplast electron transport ($J_g$) and the $J_g$ per ATP synthase ($\delta$) content in antisense plants with a variety of $\delta$ subunit of chloroplast ATP synthase (A and C) and temperature responses of $J_g$ and the $J_g$ per Rieske FeS content in antisense plants with a variety of Rieke FeS content (B and D). Three groups were classified with respect to ATP synthase ($\delta$) content or Rieske FeS content: Rieske FeS: WT (approximately 100%), plants with intermediate Rieske FeS level (58~85%) and plants with low Rieske FeS level (27~29%); ATP synthase ($\delta$): WT (approximately 100%), plants with intermediate ATP synthase ($\delta$) level (48~75%) and plants with low ATP synthase ($\delta$) level (22~26%). Data represent means ± SE, n = 3~4.