Up-regulation of cyclic electron flow and down-regulation of linear electron flow in antisense-*rca* mutant rice

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Abstract

To investigate how excess excitation energy is dissipated in a ribulose-1,5-bisphospate carboxylase/oxygenase activase antisense transgenic rice with net photosynthetic rate (P_N) half of that of wild type parent, we measured the response curve of P_N to intercellular CO_2 concentration (C_i), electron transport rate (ETR), quantum yield of open photosystem 2 (PS2) reaction centres under irradiation (F_v'/F_m'), efficiency of total PS2 centres (Φ_{PS2}), photochemical (q_P) and non-photochemical quenching (NPQ), post-irradiation transient increase in chlorophyll (Chl) fluorescence (PITICF), and $P700^+$ re-reduction. Carboxylation efficiency dependence on C_i, ETR at saturation irradiance, and F_v'/F_m', Φ_{PS2}, and q_P under the irradiation were significantly lower in the mutant. However, NPQ, energy-dependent quenching (q_E), PITICF, and $P700^+$ re-reduction were significantly higher in the mutant. Hence the mutant down-regulates linear ETR and stimulates cyclic electron flow around PS1, which may generate the ΔpH to support NPQ and q_E for dissipation of excess excitation energy.

Additional key words: chlorophyll fluorescence; CO_2 assimilation rate; intercellular CO_2 concentration; post-irradiation transient increase in chlorophyll fluorescence; $P700^+$ re-reduction.

Introduction

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO; EC 4.1.1.39) is a key enzyme catalyzing photosynthetic and photorespiratory carbon metabolism. RuBPCO activase (RCA) is necessary to activate RuBPCO *in vivo* by removing the inhibitors from RuBPCO catalytic sites (Spreitzer and Salvucci 2002, Portis 2003). To investigate the potential importance of the amount of RCA to regulate the Calvin cycle, some transgenic plants with reduced RCA contents have been produced in different plants (Mate *et al.* 1993, 1996, Jiang *et al.* 1994, Eckardt *et al.* 1997, Hammond *et al.* 1998, Zhang *et al.* 2002). We also reported that in rice net photosynthetic rate (P_N) did not significantly decline if RCA contents were higher than 35 % of the wild type (Jin *et al.* 2004), which could be partially attributed to the larger proportion of RCA located to stroma of chloroplast (Jin *et al.* 2006). However, it remains unclear how the P_N-reduced antisense mutants regulate the dissipation of excess excitation energy if low P_N results in lesser requirement for photon energy to match CO_2 assimilation. Therefore we investigated the responses of P_N to intercellular CO_2 concentration (C_i) and of fluorescence parameters of photosystem (PS) 2 to irradiance, the post-irradiation transient increase in chlorophyll (Chl) fluorescence, and the kinetics of oxidized PS1 re-reduction to explore how the reduced RCA plant dissipates excessive photon energy.

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Abbreviations: CE – carboxylation efficiency; CEF-PS1 – cyclic electron flow around photosystem 1; Chl – chlorophyll; C_i – intercellular CO_2 concentration, ETR – electron transport rate of PS2; F_v/F_m – maximal photochemical efficiency; F_v'/F_m' – the quantum yield of open PS2 centres under irradiation; NPQ – non-photochemical quenching; P_N – net photosynthetic rate; PFD – photon flux density; PITICF – post-irradiation transient increase in Chl fluorescence; PS – photosystem; q_E – energy-dependent quenching; q_P – photochemical quenching; RCA – ribulose-1,5-bisphosphate carboxylase/oxygenase activase; RuBP – ribulose-1,5-bisphosphate; RuBPCO – RuBP carboxylase/oxygenase; WT – wild type; ΔpH – pH gradient across the thylakoid membrane; Φ_{PS2} – the efficiency of total PS2 centres.

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Materials and methods

Plants: The rice plants (*Oryza sativa* L.), the wild type (WT) cv. Zhonghua No 11 and the antisense-*rca* transgenic mutants R2, were described in detail in Jin *et al.* (2006). The mutants exhibited about half of P_N and initial RuBPCO activity, less than one third of RCA content, and the same stomatal conductance as compared to the WT plants (Jin *et al.* 2006). The plants were grown in a partially shaded greenhouse with natural sunlight during the day (maximum of 800 μmol m^{-2} s^{-1}) at the Huajiachi Campus of the Zhejiang University. The greenhouse temperature was 30±2 °C during the day and 25±2 °C at night. Middle portions of the fully expanded top leaves were used for all measurements approximately seven weeks after transplanting.

CO_2 response curve and Chl fluorescence were measured using a portable photosynthesis system (*LICOR-6400*; *LICOR*, Lincoln, NE, USA) with an integrated fluorescence fluorometer (*LI-6400-40* leaf chamber fluorometer) at 30 °C set in leaf chamber. A series of reference CO_2 concentrations for CO_2 response curves was set up by the *LI-COR* CO_2 injection system (Huang *et al.* 2004) with a photon flux density (PFD) of 1 200 μmol(photon) m^{-2} s^{-1}. During the measurement of Chl fluorescence, a series of reference PFD was employed with a constant CO_2 concentration of 380 μmol mol^{-1}. The F_0 and F_m were measured after 30 min of dark-adaptation. The variable fluorescence (F_v) was calculated as ($F_m - F_0$). The dependence of the gas exchange and Chl fluorescence parameters on irradiance during a 16-min irradiation period was analyzed *in vivo* according to Rosenqvist (2001). The efficiency of PS2 centres [Φ_{PS2}; $(F_m' - F_s)/F_m'$], the quantum yield of open PS2 centres under irradiation $(F_m' - F_0')/F_m'$, the photochemical quenching [q_P; $(F_m' - F_s)/(F_m' - F_0')$], and the non-photochemical quenching [NPQ; $F_m/F_m' - 1$] were calculated from measured parameters (Genty *et al.* 1989). The electron transport rate of PS2 (ETR) was calculated as $\Phi_{PS2}\ f\ I\ a_{leaf}$ (Bilger and Björkman 1990), where I is incident PFD, f is the fraction of absorbed quanta that is used by PS2 (0.5 for C_3 plants), and a_{leaf} is the leaf absorbance, which was assumed to be 0.85 for C_3 plants (Huang *et al.* 2004). The kinetics of NPQ formation and relaxation were obtained by measuring the Chl fluorescence using the same leaves. The actinic irradiation varied between 0 and 2 000 μmol m^{-2} s^{-1}, and the photon-saturated pulses were administered at intervals of 60 s. The energy-dependent quenching (q_E) was calculated using Stern-Volmer type equation according to Thiele *et al.* (1997). For this method, the fast-relaxing component (with the first 10 min of dark relaxation after irradiation) was assigned to q_E. The post-irradiation transient increase in Chl fluorescence (PITICF) was recorded according to the procedure of Martín *et al.* (2004).

Redox changes of P700 were monitored by absorbance changes at 820–830 nm measured using a *PAM-101* Chl fluorometer (*Walz*, Effeltrich, Germany) in combination with an *ED-P700-E* emitter-detector unit. Excitation was provided by a 2-min period of far-red irradiation (>705 nm, 6 μmol m^{-2} s^{-1}) as described by Burrows *et al.* (1998) and Klughammer and Schreiber (1998). All measurements were made at 30 °C and were repeated at least six times using different plants.

Statistical methods: Statistical treatments of the data were performed by Student's *t*-test for comparison of means. For these analyses we used *SPSS 10.0* software (*SPSS*, Chicago, IL, USA) and set the statistical significance level at $p<0.05$.

Results

Carboxylation efficiency: The antisense-*rca* mutant had a very low P_N, even at the elevated CO_2 concentration (Fig. 1). The curves of response of P_N to C_i showed great difference between the mutant and the WT. The former rose linearly up to C_i near to 1 000 μmol mol^{-1} whereas the latter rose only up to less than 400 μmol mol^{-1}. Upon C_i over 800 μmol mol^{-1} the CO_2 saturation of P_N appeared in the WT, but the saturation irradiance in the mutant was more than 1 000 μmol mol^{-1}. The initial slope of P_N *versus* C_i was significantly lower in the mutant than in the WT, which meant that the carboxylation efficiency in the former was much lower than that in the latter.

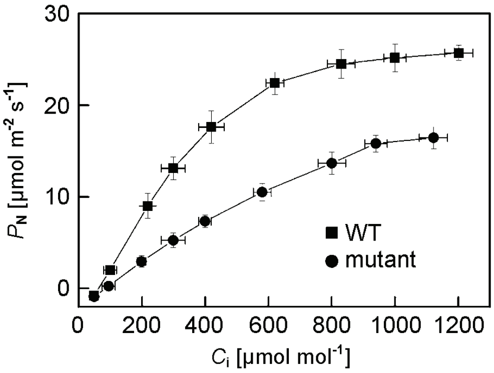

Fig. 1. The response curve of the net photosynthetic rate (P_N) to the intercellular CO_2 concentrations (C_i) in the wild type (■) and antisense-*rca* mutant (●) at irradiance of 1 200 μmol m^{-2} s^{-1}.

Chl fluorescence parameters and ETR of PS2: Except in darkness or under extremely low PFD, all fluorescence parameters of the mutant were significantly lower

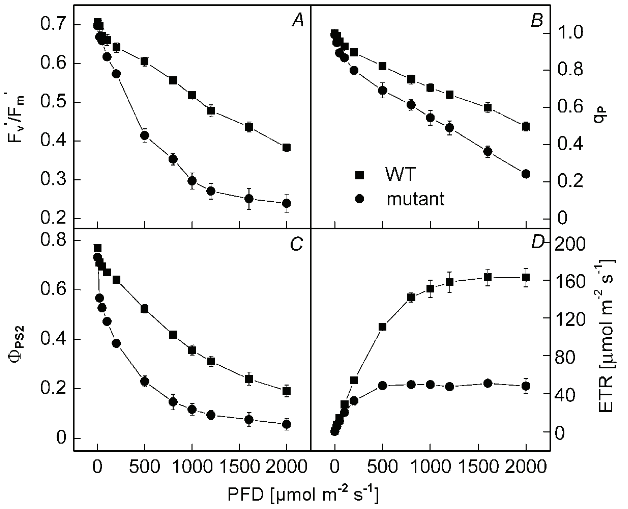

Fig. 2. The change in irradiance dependence of chlorophyll fluorescence parameters in the leaves of wild type (■) and antisense-*rca* mutant (●). *A* – the PS2 trapping efficiency (F_v'/F_m'); *B* – photochemical quenching (q_P); *C* – quantum yield of PS2 (Φ_{PS2}); *D* – relative electron transport rate (ETR).

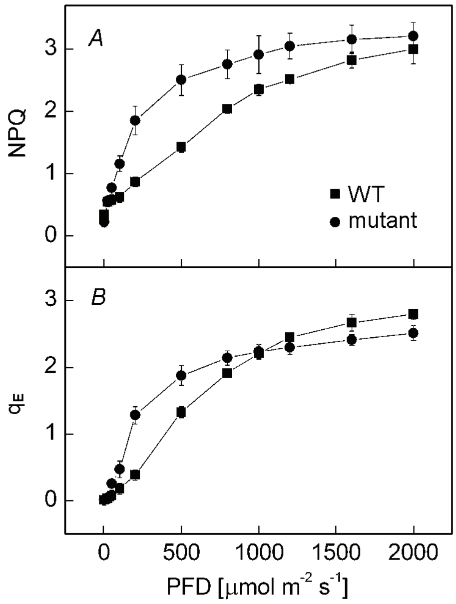

Fig. 3. The change in total NPQ (*A*) and its q_E component (*B*) in the leaves of wild type (■) and antisense-*rca* mutant (●) as a function of PFD.

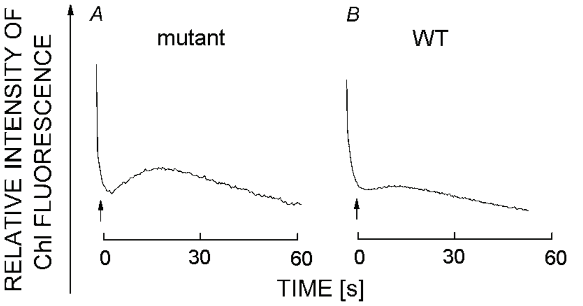

Fig. 4. Analysis of the post-irradiation transient increase kinetics of chlorophyll fluorescence after interruption of actinic irradiation in the leaves of antisense-*rca* mutant (*A*) and wild type (*B*). The curves indicate the transient increase in chlorophyll fluorescence after 5 min of "actinic light" (300 μmol m^{-2} s^{-1})

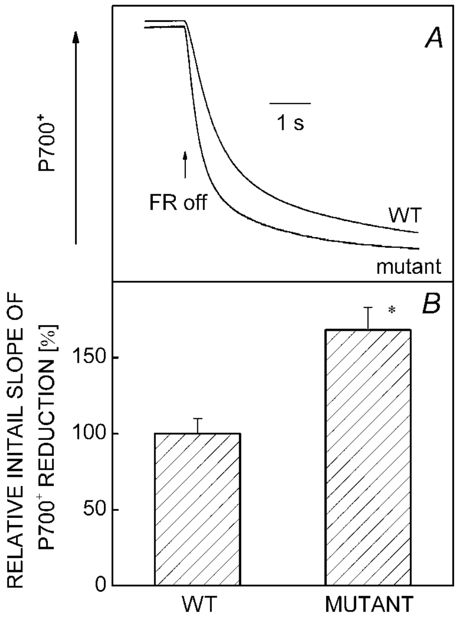

Fig. 5. The kinetics (*A*) and the relative initial slope (*B*) of P700$^+$ dark re-reduction after turning off the far-red radiation (FR) (>705 nm) in the leaves of wild type (WT) and antisense-*rca* mutant plants. The significant difference between the mutants and the WT is indicated by * for $p<0.05$.

($p<0.05$; Fig. 2) at the given PFD. Not only F_v'/F_m' (Fig. 2*A*), but also q_P (Fig. 2*B*) and Φ_{PS2} (Fig. 2*C*) exhibited a significant decrease with increasing irradiance. The declining rates of all parameters were much more rapid in the mutant than in the WT. The ETR was near saturation at 1 000 μmol m^{-2} s^{-1} in the WT, but was saturated at *ca.* 500 μmol m^{-2} s^{-1} in the mutant, which was only 30 % of the maximum ETR in the WT (Fig. 2*D*). All the parameters in the dark-adapted state suggested that the PS2 activity of the mutant was similar to that of the WT. However, the variations of all parameters and of ETR measured at PS2 with increasing PFD indicated lower requirement for assimilating power in the mutant compared to WT, resulting from the lower P_N (Figs. 1 and 2).

There was no significant difference in maximum NPQ between the mutants and the WT under the highest PFD, but the mutant had already significantly elevated NPQ at moderate PFDs (Fig. 3*A*). The assessed contribution of q_E to the total quenching effect using dark relaxation kinetics showed that q_E was increasing faster in the mutant than in the WT at PFD below 1 000 μmol m^{-2} s^{-1}.

This increase lasted no longer upon PFD over that value in the mutant, whereas it continued in the WT (Fig. 3*B*).

PITICF and P700$^+$ reduction: In comparison with the WT, the PITICF after the offset of "actinic light" was more pronounced (Fig. 4), and a faster rate of P700$^+$ reduction was found in the mutant (Fig. 5), indicating that cyclic electron flow around PS 1 (CEF-PS1) is enhanced by RCA concentration reduction. The P700$^+$ reduction after removal of "actinic light" is taken as a measure of the rate of CEF-PS1 (Burrows *et al.* 1998, Thomas *et al.* 2001, Ivanov *et al.* 2005). Thus the capacity of CEF-PS1 was enhanced in the antisense-*rca* mutant.

Discussion

As reported by many researchers, the heavily reduced-*rca* content mutants have low P_N (Mate *et al.* 1993, Jiang *et al.* 1994, Hammond *et al.* 1998, Zhang *et al.* 2002, Jin *et al.* 2004, 2006). The response curve of P_N to C_i showed that rice antisense-*rca* had only half of P_N of WT at C_i below 400 µmol mol^{-1} (Fig. 1), which means low photosynthetic capacity in the mutant. The reduction of P_N of mutant results in low requirement of excitation energy, and it may in turn inhibit further non-cyclic photosynthetic electron transport. The dependence of F_v'/F_m', Φ_{PS2}, q_P, and ETR to PFD in the mutant indicated that they more rapidly fell with increase in irradiance (Fig. 2), which confirms that non-cyclic photosynthetic electron passing through PS2 was reduced. Because no difference was found in the dark-adapted state between mutant and WT, we conclude that lower carbon assimilation causes lower linear electron transport rather than the opposite.

How does the mutant dissipate the excess excitation photon energy when q_P is inhibited? That NPQ in the mutants was much higher under medium "actinic light" than that in the WT indicated that the increase in NPQ was the main pathway to dissipate the excessive energy. Furthermore, NPQ was mainly attributable to q_E (Fig. 3). Increase in q_E and induction of thermal dissipation depend on higher ΔpH (Shikanai *et al.* 2002).

On one hand, ΔpH was built up by feedback of ATP and NADP accumulation because their consumptions were slow in the antisense-*rca* mutants. The lower q_P values in the mutant (Fig. 2) implied higher reduced states of Q_A and of the plastoquinone (PQ) pool. However, the reduction of ETR to one third of that of the WT under irradiance of 1 200 µmol m^{-2} s^{-1} (Fig. 2*D*), and the reduction of P_N to half of that of WT at C_i of 200−400 µmol mol^{-1} (Fig. 1, Jin *et al.* 2006) showed that the decrease in the rate of linear electron transport was much greater than that of P_N in the mutant. Therefore, it is unlikely that the greater ΔpH in the mutant results from ATP and NADP accumulation only. The greater ΔpH and more reduced PQ must be caused by additional factors. On the other hand, ΔpH can be formed as a result of CEF-PS1. The post-irradiation fluorescence after the offset of "actinic light" is thought to arise from the reduction of PQ by NADPH or other reducing substances accumulated in leaves under irradiation (Shikanai *et al.* 1998, Peltier and Cournac 2002). In higher plants and cyanobacteria, these processes involve mainly the CEF-PS1 (Peltier and Cournac 2002, Deng *et al.* 2003, Munné-Bosch *et al.* 2005). The higher amplitude of the post-irradiation fluorescence in the mutant suggests that the reduced RCA plants have enhanced CEF-PS1. The faster P700$^+$ re-reduction in the mutant after turning off the "actinic light" confirms that CEF-PS1 increases in the mutant. CEF-PS1 may be responsible for enhanced proton pumping and is involved in energy dissipation when CO_2 availability is reduced (Burrows *et al.* 1998, Shikanai *et al.* 2002, Munekage *et al.* 2004). Our results show that CEF-PS1 plays an important role in the production of ΔpH that leads to the effective dissipation of excess excitation energy in the mutant. Therefore, in the antisense-*rca* mutant cyclic electron flow around PS1 is enhanced for dissipation of increased excess of excitation energy, while non-cyclic electron flow is depressed. This favours plant survival under high irradiance.

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