

Quantitative Analysis of Cyclic Electron Flow in Rice Plants (*Oryza sativa* L.) Lacking PsbS Protein of Photosystem II

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In the natural environment, when plants receive excess light energy than that can be utilized in driving photosynthesis, devise different short and long-term mechanisms for photoprotection from harmful effects of high light stress. Non-photochemical quenching (NPQ) of the chlorophyll fluorescence is one of the short-term responses to high light stress that operates in the plants to protect them *in vivo*. But, under the conditions in which plants are deficient of NPQ, what is the alternative mechanism that takes the role as photoprotective means is a study of interest. Besides NPQ, other alternative pathways of photoprotection are there and one among them is the cyclic electron transport around PSI. In the present study, we evaluate the role of cyclic electron transport by quantifying its contribution to the overall process of electron transport and photoprotection using rice plants which lack energy-dependent part of NPQ (qE). It was found that efficiency of the cyclic electron flow around PSI was higher in rice T-DNA inserted plants (OsPsbS-KO), while there was no difference in the linear electron flow. In the absence of qE, cyclic electron flow around PSI can be as alternative pathway for protection from excess energy absorbed by C₃-plants. The relationship between cyclic electron flow around PSI and NPQ is discussed. Since there is the availability of mutant plants lacking of NPQ or cyclic electron transport which are sensitive to photoinhibitory illumination, these plants provide a tool for investigating into the role of different pathways.

Keywords: photosystem I, cyclic electron flow, non-photochemical quenching, PsbS protein, photosynthesis

INTRODUCTION

Sunlight is the main energy source on Earth which is energy used by photosynthesis to convert light energy to chemical energy. In nature, sometimes light intensity exceeds the capacity of the linear electron transport of the chloroplasts of higher plants. Therefore, plants have developed adaptive mechanisms to control the efficiency of utilization of the energy and photosynthetic electron transport (Chow, 1994; Osmond, 1994). These photoprotective mechanisms are classified as either long-term or short-term responses. The long-term responses include avoidance mechanisms that involve changes in the orientation of leaves (Björkman and Demmig-Adams, 1994) or chloroplasts (Park et al., 1996) and modulation of the composition of the photosynthetic apparatus by light acclimation (Anderson and Osmond, 1987). The most prominent short-term response is non-photochemical quenching (NPQ), which plays an important role in the photoprotection of photosystem (PS) II *in vivo*. NPQ is subdivided into three components according to their relaxation kinetics in darkness

following a period of illumination, as well as their responses to various inhibitors. The slowest component of NPQ is qI, which is related to photoinhibition or the slowly reversible damage to PSII reaction centers (Osmond et al., 1997). The second component of NPQ is qT, which reflects the phosphorylation-mediated migration of light-harvesting complex (LHC) II between PSII and PSI (state transition) (Harrison and Allen, 1993). The fastest and the most important component of NPQ is qE, the energy-dependent quenching. This component depends on three major parameters: the development of transthylakoid proton gradient (Δ pH), the amount of pigments involved in xanthophyll cycle, and the existence of a PsbS subunit in PSII (Müller et al., 2001). qE is characterized by: (1) its sensitivity to uncouplers of the proton gradient (Oxborough and Horton, 1988) and, inhibition by N,N'-dicyclohexylcarbodiimide, an inhibitor of protonation of protein residues (Walters et al., 1994), (2) the light-induced absorbance changes at 535 nm (Ruban et al., 1993), (3) the shortening of the lifetime of a specific chlorophyll (Chl) fluorescence component from ~2.0 to ~0.4 ns (Gilmore et al., 1995)

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and (4) carotenoid cation radical formation (Holt et al., 2005).

Since the NPQ may reduce the efficiency of photosynthesis under low-light conditions regulated by monitoring of the transthylakoid proton gradient (ΔpH). In such kind of circumstances, alternative electron transport pathways, such as the cyclic electron transport within PSII, the cyclic electron flow around PSI and the water-water cycle, may regulate the generation of NPQ. The cyclic electron flow around PSI and water-water cycle regulates the NPQ by modifying the rate of ΔpH generation through ATP balance (Heber and Walker, 1992; Asada, 1999). The ATP pool is one of the main parameters that control the NPQ and the rate of electron flow through the Benson-Calvin cycle. Although cyclic electron flow around PSI was discovered several decades ago (Arnon et al., 1954), its role, especially in C_3 -plants was controversial. After the discovery set of the *Arabidopsis thaliana* mutants, *pgr* and *crr* (Munekage and Shikanai, 2005), the role of cyclic electron flow around PSI, as well as the different routes for cyclic electron flow with participation of the *cyt b₆f* complex, plastoquinone, plastocyanin, and NDH complex, have been highlighted. The plants lacking either energy-dependent quenching of chlorophyll fluorescence (*npq4-1* (Li et al., 2000); *npq1-2* (Niyogi et al., 1998) of *Arabidopsis*; PsbS-KO rice plants (Koo et al., 2004)) or defective in cyclic electron flow around PSI (*pgr1* (Munekage et al., 2001); *pgr5* (Munekage et al., 2002) and *crr2-4* (Munekage et al., 2004)) are sensitive to high light stress.

Therefore, in the present study, we investigated the efficiency of the cyclic electron flow around PSI in both wild-type (WT) and qE-less (OsPsbS-KO) mutant rice plants. We found that efficiency of the cyclic electron flow around PSI is higher in OsPsbS-KO plants, while there is no difference in the linear electron flow.

MATERIALS AND METHODS

One-month-old seedlings of WT and OsPsbS-knockout mutant rice (*Oryza sativa* L.) plants (Munekage et al., 2004) were grown in soil in a greenhouse under sunlight at a temperature of $28 \pm 2^\circ C$. The oxidation and re-reduction of P_{700} in rice leaves were determined with a dual wavelength (820/870 nm) unit (ED-p700DW) attached to a phase amplitude modulation fluorometer, PAM101/102 (Walz, Effeltrich, Germany). To obtain the steady-state signal was reached by applying far-red light ($12 \mu mol photons m^{-2} s^{-1}$, peak wavelength 715 nm). The maximum signal amplitude at end of actinic light excitation (white or green light) was taken as the total amount of photo-oxidizable P_{700} .

To measure the re-reduction kinetics and to calculate half-lifetime of the oxidized P_{700} data obtained after switch of the actinic light. Data transformed by Data Acquisition System NI-DAQ Model USB-6009 (National Instruments, Australia) by rate 100 Hz.

Infiltration of the chemicals into rice leaf segments was done as described (Kim et al., 2002).

RESULTS AND DISCUSSION

It has been evidenced that cyclic electron flow has an important role on the protection against photoinhibitory illumination (Munekage et al., 2004). In *pgr5* mutant of *Arabidopsis thaliana* plants defective in cyclic electron flow around PSI non-photochemical quenching (NPQ) of chlorophyll fluorescence also decreased (Munekage et al., 2002). Because the both processes participate in photoprotection mechanism, hence to know the relation between NPQ and cyclic electron flow around PSI we measured cyclic electron flow in WT and PsbS-KO rice plants.

We used detached rice leaves infiltrated with different chemicals to switch between cyclic and linear electron transport pathways. Control detached dark-adapted WT and PsbS-KO rice leaves were infiltrated with 150 mM sorbitol to prevent chloroplasts from osmotic shock (Joliot and Joliot, 2005). Far-red illumination during 10 s which leads to P_{700} oxidation was much slower (Figure 1) in the leaves of both WT and PsbS-KO rice plants showing that cyclic electron flow around PSI operate very slowly. In other experiment we infiltrated detached dark-adapted WT and PsbS-KO rice leaves with 150 mM sorbitol plus 1 mM methyl violologen (MV) to switch linear electron transport pathway only, because MV accept electrons from all PSI. Far-red illumination leads to P_{700} oxidation occurs less than 4 s due to direct electron transfer from P_{700}^+ to MV. In regards to the linear electron transport pathway we did not observed any differences between WT and PsbS-KO rice plants confirming our previous observation on whole chain electron transport rate (Zulfugarov et al., 2007). The remarkable difference were observed when the electron transport pathway switched to cyclic mode by infiltration with 150 mM sorbitol plus 40 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) plus 2 mM hydroxylamine of dark-adapted WT and PsbS-KO rice leaf segments.

Although kinetics of P_{700} oxidation were slower with compared MV infiltrated leaf segments in both WT and PsbS-KO plants (Figure 1), in PsbS-KO plants it was much more slower and even did not completed after 20 s (data not shown). Accord-

ing to Joliot and Joliot (2005) the slow oxidation of P_{700} , in such kind of conditions where no artificial electron donor, is due to an efficient recycling of electrons to P_{700} via the cyclic electron flow around PSI. It has also been proposed that leakage of electrons is from cyclic electron flow to oxygen (Mehler reaction) or to the Benson-Calvin cycle (Joliot and Joliot, 2002, 2006).

Illumination of dark-adapted leaves of WT and PsbS-KO plants with 30 s far-red light leads to oxidation of P_{700} (Figure 1), while termination of illu-

mination resulted in re-reduction of oxidized P_{700} . Re-reduction rate of oxidized P_{700} allow us to estimate cyclic electron flow around PSI in C_3 plants, too (Fan et al., 2007). Figure 2 shows the results of the re-reduction of oxidized P_{700} in WT and PsbS-KO plants under same conditions as in Figure 1. In control leaves infiltrated with 150 mM sorbitol after turn off far-red illumination oxidized P_{700} rapidly reduced. Re-reduction rate of oxidized P_{700} in MV and DCMU infiltrated leaves of WT and PsbS-KO rice plants was relatively slow (Figure 2).

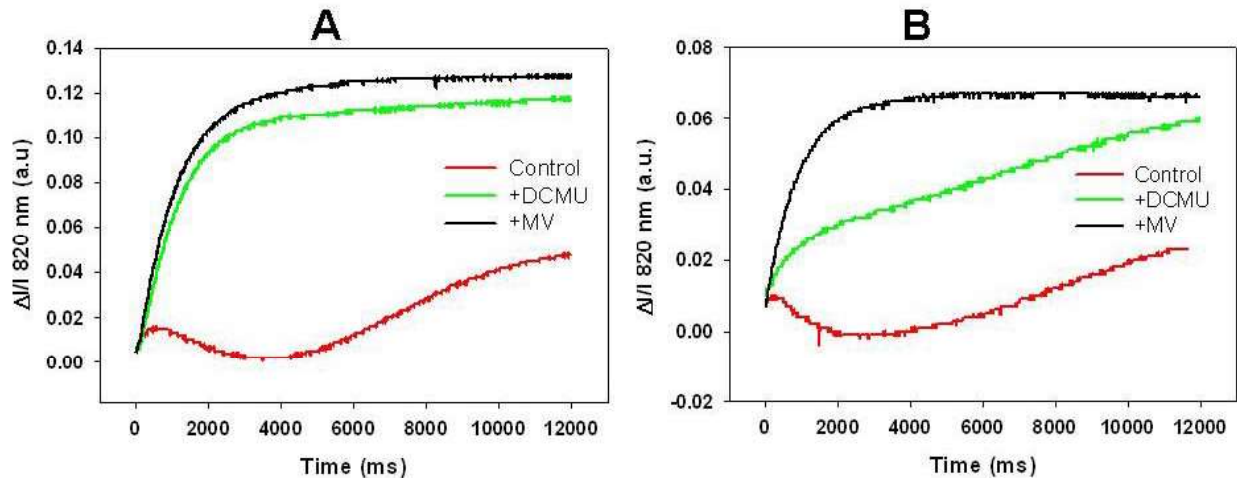


Figure 1. Absorption changes due to oxidation of P_{700} measured at 820 nm induced by far-red illumination of dark-adapted leaves of rice WT (A) and PsbS-KO (B) plants. Red – control leaves were infiltrated with 150 mM sorbitol only; Green – Leaves were infiltrated with 150 mM sorbitol plus 40 μ m DCMU plus 2 mM hydroxylamine; Black – Leaves were infiltrated with 150 mM sorbitol plus 1 mM MV. The experiments were repeated at least for three times and the representative curves shown.

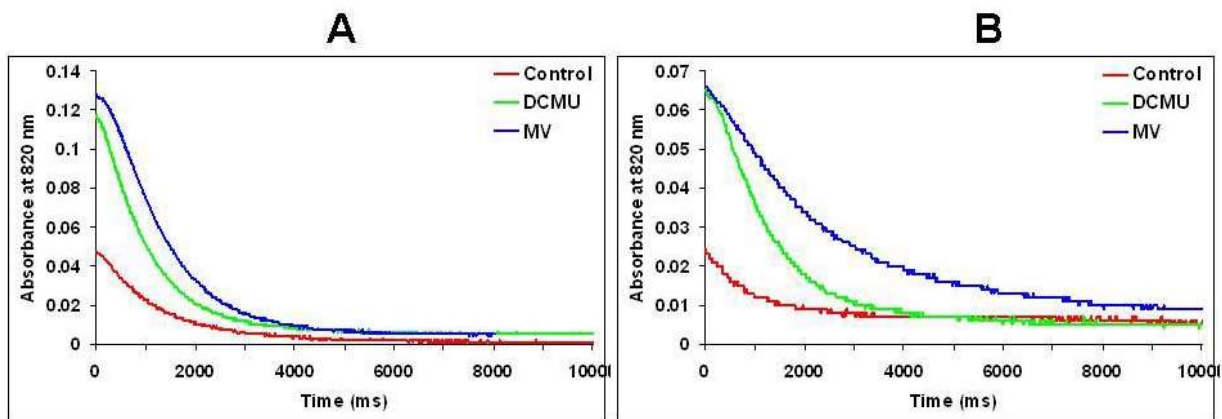


Figure 2. The re-reduction kinetics of oxidized P_{700} measured as absorption changes at 820 nm after termination of far-red illumination of dark-adapted leaves of rice WT (A) and PsbS-KO (B) plants. Red – control leaves were infiltrated with 150 mM sorbitol only; Green – Leaves were infiltrated with 150 mM sorbitol plus 40 μ m DCMU plus 2 mM hydroxylamine; Black – Leaves were infiltrated with 150 mM sorbitol plus 1 mM MV. The experiments were repeated at least for three times and the representative curves are shown.

We determined the contribution of cyclic electron flow around PSI in WT and PsbS-KO plants in dark-adapted condition where Benson-Calvin cycle

is fully deactivated and by applying the green light to activate Benson-Calvin cycle. The kinetics of P_{700} oxidation determined by switching off the

green light for a 10 s and applying a far-red light excitation at indicated time (Figure 3). The curve in black shows the kinetics of P_{700} oxidation after 20 min green light illumination to fully activate the Benson-Calvin cycle which dark-adapted for 2 min before measuring the P_{700} oxidation kinetics. It can be seen that with activation of Benson-Calvin cycle (green light illumination) the amount of the oxidized P_{700} increases in both WT and PbsS-KO rice plants. But there are differences between WT and PbsS-KO rice plants. So, we have calculated the efficiency of the cyclic electron flow around PSI in WT and PbsS-KO rice plants during activation of Benson-Calvin cycle. To calculate the efficiency of the cyclic electron flow around PSI the re-reduction kinetics of oxidized P_{700} after turn off the 10 s far-red light for each time point were analyzed. The half-life time of reduction of the oxidized P_{700} were plotted against the illumination time. Figure 4 displays the relative amount of the cyclic electron flow in WT and PbsS-KO rice plants.

It is interesting that during the firsts minutes of illumination when occurs a partial oxidation of P_{700} (Harbinson and Hedley, 1993; Joliot and Joliot, 2006) there was no difference between WT and

PbsS-KO rice plants in the efficiency of the cyclic electron flow around PSI. Although, later when Benson-Calvin cycle activated more and the oxidized P_{700} decreased the efficiency of the cyclic electron flow around PSI differed remarkably. Thus, our data in agreement with findings (Golding and Johnson, 2003) that the inactivation of Benson-Calvin cycle by dark-adaptation which plants lacks CO_2 is associated with an increase rather than a decrease in oxidized P_{700} concentration. As suggested in (Joliot and Joliot, 2005) the dark-adapted leaf of C_3 -plants includes 30% of the cyclic electron flow around PSI and 70% of the linear electron flow. The data in Figure 4 fitted to these values for WT rice plants, but in the case of PbsS-KO rice plants shows different values; 40% of the cyclic electron flow around PSI and 60% of the linear electron flow.

Of course we can not rule out the other possibilities, because a part of the slow oxidation of P_{700} is associated with the oxidation of the ferredoxin or by oxygen via Mehler reaction (Johnson, 2005; Joliot and Joliot, 2006). It has been suggested that the concentration of ATP has a crucial role on the formation of the cyclic electron flow around PSI (Crowther et

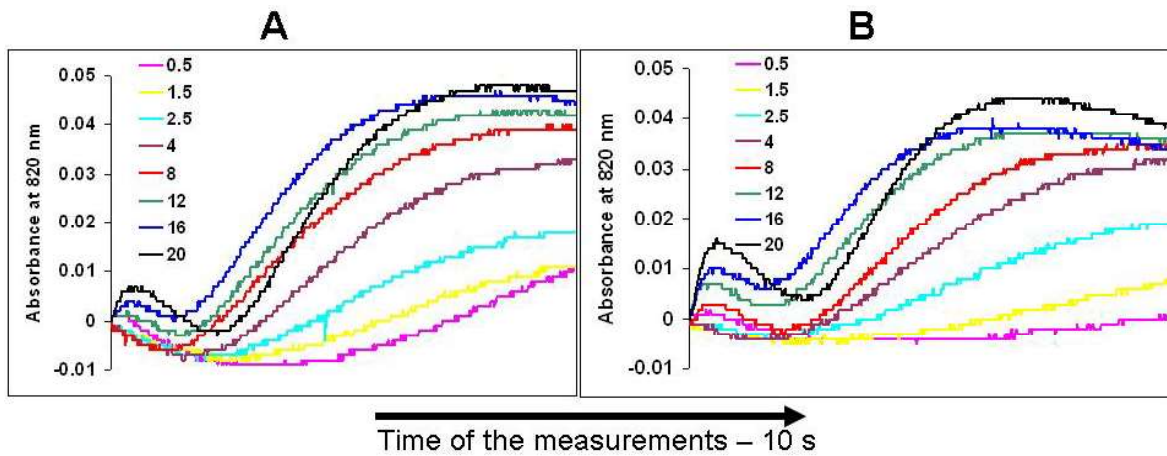


Figure 3. The oxidation of P_{700} measured as absorption changes at 820 nm induced by far-red illumination measured at different times of green-light illumination. Digits in the figure green light illumination time in minutes. The experiments were repeated at least for three times and the representative curves shown.

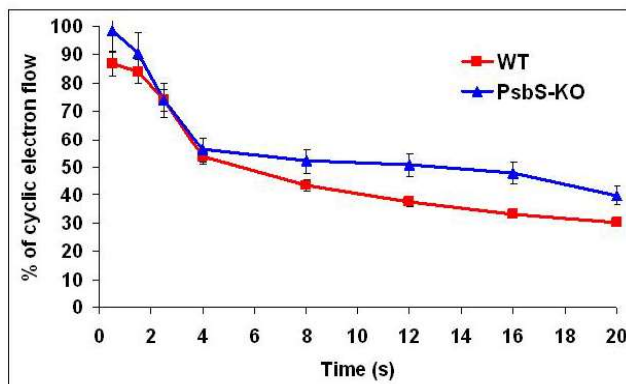


Figure 4. Efficiency of the cyclic flow around PSI determined as a function of the time of green light illumination. Same experimental condition as in Figure 3.

al., 1979). It has also been proved that proton motive force (Avenson et al., 2005) play a vital role on the generation of the energy-dependent (qE) quenching of chlorophyll fluorescence and ATP synthesis. Taken together these data indicate that these two processes, cyclic electron flow around PSI and energy-dependent quenching of chlorophyll fluorescence have very close relations. Role of the different routes of cyclic electron flow around PSI and quantitative analysis of cyclic electron flow around PSI under conditions were no limitation of the Benson-Calvin cycle under investigation. Thus, the increased efficiency of the cyclic electron flow around PSI may function as alternative protection mechanism against photoinhibitory illumination when the NPQ is much less. It has been shown that in *pgr5* mutant of *Arabidopsis thaliana* plants which defective in the cyclic electron flow around PSI NPQ level also become less and these plants do not grow well under high light (Munekage and Shikanai, 2005). Despite a large number of the observations reported so far, but there is no clear explanation for the role of the energy-dependent quenching in photoinhibition (Zulfugarov et al., 2005) and its relations with cyclic electron flow around PSI (Munekage and Shikanai, 2005). Also, the relationship between different routes of cyclic electron flow around PSI is unclear at present (Endo et al., 2008). Development of a method for quantification of each routes of cyclic electron flow around PSI separately and its relation to linear electron transport and NPQ is necessary for understanding the relationship between these processes. Although it is widely accepted that PSII is the primary site of photoinhibition (Aro et al., 1993), photoinhibition of PSI also occurs even under low light intensities when it combined with chilling (Soinike, 1996; Kim et al., 2005). The exact process of photoinhibition of PSI in the absence of cyclic electron flow around PSI remains unclear. The energetic imbalance of overall photosynthesis due to lack of cyclic electron flow around PSI, most probably, causes over-reduction of the stroma, which directly triggers PSI photoinhibition. It might be the reason why in the plants lacking energy-dependent-quenching cyclic electron flow around PSI is activated.

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