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Research article

Abolition of photosystem I cyclic electron flow in *Arabidopsis thaliana* following thermal-stress

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ABSTRACT

Heat tolerance of Arabidopsis thaliana (WT) and its mutants, *crr2-2*, lacking NADPH-dehydrogenase (Ndh-pathway), and *pgr5*, deficient in proton gradient regulation and/or ferredoxin-quinone-reductase (FQR-pathway), was studied from 30 to 46 °C. Chlorophyll fluorescence revealed that thermal damage to photosystem II (PSII) was maximal in WT plants following short-term exposure of leaves to moderate or high temperature stress. Thermal stress impaired the photosynthetic electron flow at oxidizing and reducing sides of PSII. This was deduced from the transformation of temperature dependent OJIP to OKP patterns, changes in the relative amplitudes of K-step fluorescence rise and F_v/F_o ratio. The amplitude of the K-peak that corresponds to the magnitude of damage to the oxygen evolving complex (OEC) in *crr2-2* mutants was about 50% of that observed in WT plants exposed to 46 °C. The damage to OEC in *pgr5* mutants was relatively smaller and thus their PSII complexes were more heat tolerant. P700 oxidation–reduction kinetics following heat-stress revealed that photosystem I (PSI) complexes remained oxidizable either with 10-ms multiple turn-over flashes or far-red illumination but the complementary cyclic electron flow around PSI (CEF) was abolished in both mutants. With further increase in incubation temperature, CEF was fully suppressed even in WT. Thus, P700 turn-over was not enhanced following thermal stress. Furthermore, the experimental data predicts the onset of pseudocyclic electron transport with molecular oxygen as terminal acceptor in *crr2-2* and *pgr5* mutants but not in wild type Arabidopsis subjected to severe thermal-stress.

1. Introduction

Photosynthesis, the most important physiological processes in plants, is very sensitive to heat stress [1]. According to [2,3], the photosynthetic machinery is subjected to significant changes in its structure and function upon exposure to severe or mild heat stress including an altered lipid and protein composition of the thylakoid membranes. Until date, a general consensus is that the photosynthetic apparatus has at least three major sensitive sites for heat-stress. These are i) the membrane bound multimeric photosystem II (PSII) with its oxygen-evolving complex and photosystem I (PSI), ii) the ATP generating and iii) carbon assimilation processes [4]. The action of heat stress on PSII has been the subject of intensive research since it is the most thermosensitive component of the photosynthetic apparatus [3,5–9]. However, there is little information available concerning the heat induced changes in the flux of e^- passing through the PSI complex.

Three major routes of photosynthetic e^- transport involving PSI are functional in cyanobacteria, eukaryotic algae, and chloroplasts of higher plants. These are the non-cyclic or linear e^- flow (LEF) from water to $NADP^+$ in which the e^- are mobilized in both photosystems through the membrane bound Cyt b_6/f complex and the mobile e^- carriers such as plastoquinone (PQ), plastocyanin (PC), and ferredoxin (Fd). The second pathway is the pseudocyclic e^- flow or water–water cycle (PET) in which the photosystems are operational in series but molecular oxygen acts as a terminal e^- acceptor. In the third route, the e^- are cycled through PSI alone [10] and referred as cyclic e^- flow around PSI (CEF). NADPH can be generated solely

Abbreviations: AL, actinic light; Chl, chlorophyll; CEF, cyclic electron flow; Cyt b_6/f, cytochrome b_6/f; e^-, electron; ETR, electron transport rate; F_A, F_B, and F_X, iron–sulfur centers; Fd, ferredoxin; FI, fluorescence induction; FNR, ferredoxin-NADP oxidoreductase; FQR, ferredoxin-plastoquinone reductase; FR-light, far-red light; LEF, linear electron flow; MT-flash, multiple turn-over white flash; MV, methylviologen; Ndh, chloroplast NAD(P)H dehydrogenase; NPQ, non-photochemical quenching; OEC, oxygen-evolving complex; P680, special chlorophyll(s) acting as the primary electron donor of photosystem II; P700, reaction center chlorophyll of photosystem I; PC, plastocyanin; PEF, pseudocyclic electron flow; PQ, plastoquinone; PS, photosystem; Q_A and Q_B, primary and secondary electron acceptor of PSII, respectively; qE, energy-dependent quenching; qN, nonphotochemical quenching; ROS, reactive oxygen species; SP, saturating white light pulse; ST-flash, single turn-over flash; $t_{1/2}$, half-time; Tyr_Z, Tyr-161 of the D_1 protein.

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through the LEF. Whereas ATP synthesis is coupled to all the three routes involving Cyt b_6/f complex that generates ΔpH following proton translocation across the thylakoid membrane.

The ability of plants to shift the mode of photosynthetic e^- flow between LEF and CEF is essential to maintain the ATP/NADPH ratio that permits the prompt adjustment to metabolic changes and stress conditions [11]. Two types of CEF are functional in plants [11–14]. In one of the pathway, the e^- are recycled from NADPH to the PQ-pool and then to oxidized P700 (Fig. 1). This pathway is mediated by a large multimeric complex, the NAD(P)H dehydrogenase (Ndh), which is membrane bound and proximal to PSI [11,15]. The Ndh-dependent CEF is essential for additional ATP synthesis during stress conditions and is also involved in chlororespiration in the dark [16–18]. Recently, *crr2-2*, a chlororespiratory mutant of Arabidopsis was developed in which the processing of the mRNA of *ndh B* is being impaired [19]. This generally leads to the low accumulation of Ndh and restriction in its functions in vivo [19].

In the other route of CEF around PSI, the e^- are transferred from Fd which is photoreduced at the stromal side, to the Cyt b_6/f complex and subsequently to PQ. This pathway involves an antimycin A sensitive enzyme, the ferredoxin-quinone oxidoreductase (FQR), as depicted in Fig. 1. With identification of the Arabidopsis mutant deficient in non-photochemical quenching (NPQ) of the absorbed light energy owing to the lack proton gradient regulation-5 (*pgr5*) protein and also Fd-dependent CEF, it was originally proposed that CEF around PSI is necessary for photosynthesis [20]. However, this proposal was questioned by several others and hotly debated until date. One suggestion is that PGR5 may modulate the conductivity of ATP synthase that could affect proton motive force and NPQ [21]. In another model, PGR5 has been implicated in feedback regulation of photosynthetic electron flow by plastid redox poising [22]. It is also proposed that another transmembrane protein, PGRL1, can form a complex with the PGR5 and exerts control in shifting from LEF to CEF *in vivo* [23].

Several studies have proposed that components of Ndh- and FQR-pathways play a vital role in photoprotective or long-term acclimation of plants to external cues and have been the subject of intense research until date. Therefore, it is of our interest to verify the involvement of these pathways in addition to the functionality of LEF and the components of the lumenal side of thylakoids of Arabidopsis mutants *crr2-2* and *pgr5* (Fig. 1) when subjected to heat stress. Together with WT plants, they were subjected to short-term exposure to heat stress in darkness. Our experimental results reveal for the first time PSII centers of *pgr5* mutants are less susceptible to high temperature stress if compared to *crr2-2* mutants or WT plants. On the other hand, redox kinetics of P700 in the mutants and WT Arabidopsis revealed a temperature dependent suppression of CEF.

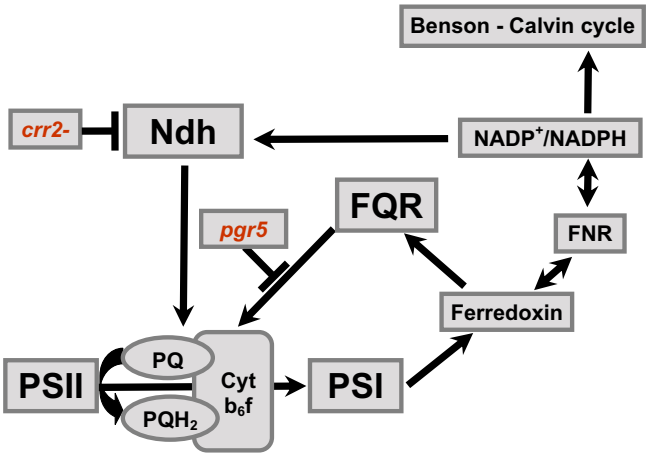

Fig. 1. A simplified scheme of the PSII, NDH and FQR mediated electron flow to PSI complexes via Cyt b_6/f complex and the probable deletion sites in *crr2-2* and *pgr5* mutants, respectively. Ndh: NAD(P)H-dehydrogenase; FNR, ferredoxin-NADP reductase; FQR, ferredoxin-plastoquinone reductase; PQ, plastoquinone; PS, photosystems. For other details, see "Introduction section".

2. Results

2.1. Chlorophyll fluorescence induction in Arabidopsis WT, crr2-2 and pgr5 phenotypes

Intact leaves of Arabidopsis WT, *crr2-2* and *pgr5*, respectively, were incubated at various temperatures ranging from 23–46 °C for 5 min followed by 30-min incubation in dark before fluorescence measurements. In leaves not subjected to heat treatment, the initial fluorescence O-level (F_o) rose to the maximum *P*-level ($=F_m$) with two intermediate steps J and I, when plotted against time on a logarithmic scale (Fig. 2, 23 °C). This polyphasic rise to J-, I-, and P-levels occurring at the time scale of ~2, 20–30, and 65–200 ms, respectively [24–26], is associated with the reduction of the quinone acceptors of PSII [27–33].

The kinetics of the fluorescence rise was unaffected by the incubation of leaves until about 38 °C regardless of the type of experimental variety used in this study. However, a further increase in the incubation temperatures above this level strongly affected the shape and kinetics of the fluorescence rise (Fig. 2) as the J–I phase

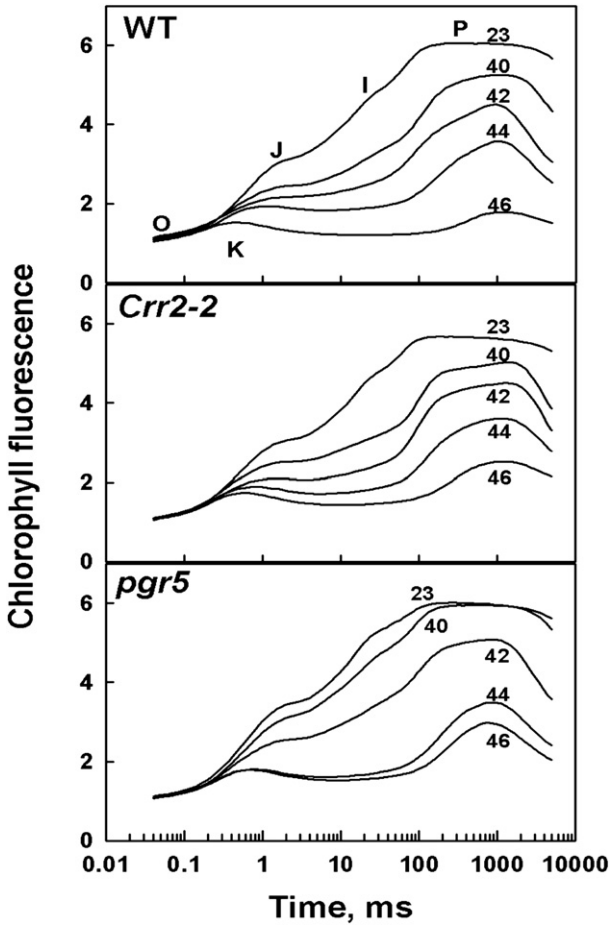

Fig. 2. Original traces of fluorescence induction in dark-adapted leaves from Arabidopsis WT, *crr2-2*, and *pgr5* in control leaves (23 °C) or in leaves subjected to 40 °C, 42 °C, 44 °C, or 46 °C for 5 min, respectively. The numbers adjacent to curves correspond to the temperature of treatment.