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ORIGINAL ARTICLE

Plastoquinone homeostasis in plant acclimation to light intensity

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Abstract
Arabidopsis plants were grown from seeds at different photon flux densities (PFDs) of white light ranging from 65 to 800 µmol photons m^{-2} s^{-1}. Increasing PFD brought about a marked accumulation of plastoquinone (PQ) in leaves. However, the thylakoid photoactive PQ pool, estimated to about 700 pmol mg^{-1} leaf dry weight, was independent of PFD; PQ accumulation in high light mostly occurred in the photochemically non-active pool (plastoglobules, chloroplast envelopes) which represented up to 75% of total PQ. The amounts of PSII reaction center (on a leaf dry weight basis) also were little affected by PFD during growth, leading to a constant PQ/PSII ratio at all PFDs. Boosting PQ biosynthesis by overexpression of a solanesyl diphosphate-synthesizing enzyme strongly enhanced the PQ levels, particularly at high PFDs. Again, this accumulation occurred exclusively in the non-photoactive PQ pool. Mutational suppression of the plastoglobular ABC1K1 kinase led to a selective reduction of the thylakoid PQ pool size to *ca*. 400 pmol mg^{-1} in a large range of PFDs, which was associated with a restriction of the photosynthetic electron flow. Our results show that photosynthetic acclimation to light intensity does not involve modulation of the thylakoid PQ pool size or the amounts of PSII reaction centers. There appears to be a fixed amount of PQ molecules for optimal interaction with PSII and efficient photosynthesis, with the extra PQ molecules being stored outside the thylakoid membranes, implying a tight regulation of PQ distribution within the chloroplasts.

Keywords Plastoquinone · Photosystem II · Light acclimation · Photosynthetic electron flow

Introduction

Plastoquinone is a major component of the photosynthetic machinery carrying electrons from photosystem II (PSII) to cytochrome *b6/f* in the thylakoid membranes of the chloroplasts (Amesz 1973; Crane 2010). In vascular plants, plastoquinone-9 (hereinafter referred as PQ) is the major form of plastoquinone (Nowicka and Kruk 2010; Havaux 2020; Nowicka et al. 2021). PSII-mediated electron flow involves a PQ molecule firmly bound to the Q_A site of the PSII protein D1 which transfers electrons from pheophytin to another PQ molecule loosely bound at the Q_B site of the D2 protein. After two-electron reduction and proton uptake from the stroma, the formed plastoquinol (PQH_2) molecule dissociates from the Q_B pocket and enters the mobile PQ pool in the thylakoid membrane. Re-oxidation of PQH_2 coupled with proton translocation to the lumen proceeds at the cytochrome *b6/f* complex. PQ is thus involved both in the intersystem electron flow and the establishment of the transthylakoidal pH gradient. Due to its central position in the PSII-to-PSI electron transport chain, PQ can also receive electrons from other sources than the acceptor side of PSII including cyclic electron flow around PSI and chlororespiration (Rochaix 2011).

PQ is present in much larger amounts than PSII, with previous estimations of the number of PQ molecules per PSII being in the range 5–20 in green plants (Forbush and Kok 1968; McCauley and Melis 1986; Ksas et al. 2018). It has been proposed that this value is modulated by the environmental conditions (e.g. light intensity) to optimize photosynthesis (Suslichenko and Tikhonov 2019). In particular, it has been suggested that, in low light, photosynthetic electron transport would be favored by an increase in the rapidly photoreducible PQ pool (Suslichenko and Tikhonov 2019). This idea appears to be in contradiction with the fact that the PQ concentration in plant leaves is

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decreased, rather than increased, in low light conditions compared to high light conditions (e.g. Boardman 1977; Szymanska and Kruk 2010; Ksas et al. 2015). However, a large fraction of the PQ pool is located outside the thylakoid membranes, principally in the plastoglobules (Zbierzak et al. 2009; Eugeni-Piller et al. 2012; van Wijk and Kessler 2017). This fraction of the PQ molecules is not involved in photosynthetic electron transport (Ksas et al. 2018). The density of plastoglobules in the chloroplasts and the amounts of PQ stored in those structures tend to increase in bright light (e.g. Espinoza-Corral et al. 2021; van Wijk and Kessler 2017). Although the function of the plastoglobular PQ molecules is still elusive, they have been shown to participate in the biosynthesis of some metabolites, such as carotenoids and phylloquinone (Carol et al. 1999; Wu et al. 1999; Eugeni-Piller et al. 2011), and to constitute spare parts for the photosynthetic electron transport chain to replace photooxidized PQ (Ksas et al. 2018).

The present study was undertaken to clarify to what extent the PQ content and localization are modulated in Arabidopsis plants acclimated in the long term to different photon flux densities (PFDs). In this context, an important aspect is to understand the role of the PQ adjustments, if any, in photosynthetic acclimation to light. PQ was quantified by liquid chromatography in the photochemically active and non-active PQ pools while PSII was quantified in parallel by biochemical and biophysical approaches. The presented results show a remarkable constancy of the size of the thylakoid PQ pool and the maintenance of a constant PQ/PSII ratio whatever the photon flux density to which plants are acclimated. The surplus of PQ is stored in a non-photoactive state.

Materials and methods

Plant material and growth conditions

Arabidopsis plants (*Arabidopsis thaliana* (L.) Heynh.) were grown in a phytotron of the Phytotec platform (BIAM Institute, CEA Cadarache) under controlled conditions of temperature (23/18 °C, day/night) and air humidity (55%). Plants were grown under short-day conditions (8-h illumination). White light supplied by halogen metal halide lamps (Osram) was adjusted to provide different PFDs: 65, 120, 250, 350, 550, 700, 750 and 800 (± 5%) µmol m^{-2} s^{-1}. Plants were grown from seeds under these different PFDs. The following Arabidopsis genotypes were used in this work: the wild type (WT, ecotype Col-0), the *abc1k1* mutant (Pralon et al. 2020) and two transgenic lines (*OE:SPS1*, L12 and L3) overexpressing the *SPS1* (*SOLANESYL DIPHOSPHASE SYNTHASE 1*) gene (Ksas et al. 2015).

Chlorophyll fluorometry

Chlorophyll fluorescence emission from attached leaves was measured with a PAM-2000 fluorometer (Walz). The actual quantum yield of PSII photochemistry (Φ_{PSII}) was calculated as $1-(Fs/Fm)$ where Fs is the steady-state chlorophyll fluorescence level and Fm is the maximal fluorescence level. Fs and Fm were measured in leaves exposed to different PFDs of white light provided by a KL-1500 Schott lamp equipped with an optic fiber. Fm was induced by a 800-ms flash of intense white light.

Chlorophyll quantification

Chlorophyll *a* and *b* were measured in 80% acetone according to Lichtenthaler and Wellburn (1983).

Plastoquinone and tocopherol analyses

PQ in the reduced or oxidized forms and α-tocopherol were measured by HPLC with UV absorbance and fluorescence detection. Leaf discs (5 discs of 0.8 cm diamater) were grinded in ethyl acetate. After centrifugation, the supernatant was filtered and evaporated on ice under a stream of N_2. The residue was recovered in methanol/hexane (17/1) and analyzed by HPLC as described elsewhere (Ksas et al. 2015, 2018). The column was a Phenomenex Kinetex 2.6 µm, 100 × 4.6 mm, 100 Å. Separation of tocopherols and PQ was done in the isocratic mode with methanol/hexane (17/1) as solvent system and a flow rate of 0.8 ml min^{-1}. Tocopherols and reduced PQ were detected by their fluorescence at 330 nm with an excitation at 290 nm. PQ in the oxidized state was measured by its absorbance at 255 nm. PQ standard was a kind gift from Dr. J. Kruk (Krakow, Poland). α-Tocopherol standard was purchased from Sigma.

Determination of the thylakoid photochemically active PQ pool

The size of the photoactive PQ pool in the thylakoid membranes was determined from the difference between the amount of reduced PQ after high light (maximal reduction of the photoactive PQ pool) and the amount measured after far-red light (maximal oxidation of the photoactive PQ pool) (Ksas et al. 2015, 2018). The photochemically inactive pool corresponds to the PQ molecules stored outside the thylakoid membranes (plastoglobules, envelopes) (Ksas et al. 2018). They are the sum of the PQ molecules remaining in the oxidized state in the leaves illuminated with the intense white light and the PQ molecules that remained reduced

in the far-red-treated leaves. This method was previously validated by comparison with the PQ amounts measured in fractionated chloroplasts (Ksas et al. 2018). Small leaf discs (0.8 cm diameter) were taken from 5-week old plants. The discs were illuminated for 10 s with saturating white light (2500 µmol m^{-2} s^{-1}) using a fiber-optic system allowing maximal reduction of the PQ pool. Samples were directly flash frozen in liquid nitrogen at the end of the light treatment while still illuminated. A second series of discs taken from the same leaves was dark-adapted for 15 min and then treated with far-red light (735 nm, 1.5 µmol m^{-2} s^{-1}) for 2 min allowing maximal oxidation of the PQ pool. Samples were then grinded in the frozen state and extracted with cold ethyl acetate. Reduced and oxidized PQ was determined by HPLC as described above.

Spectroscopic quantification of PSII content

The concentration of PSII reaction center was measured in leaves by spectroscopic methods (flash-induced electrochromic shift at 520 nm and P700 absorbance spectroscopy) using a JTS-10 spectrophotometer (BioLogic). Fresh leaves were infiltrated with 20 µM DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), 1 mM hydroxylamine in 20 mM sorbitol, and preilluminated to inhibit PSII. The full amount of P700 + was formed after 5 s of illumination and was measured based on the extinction coefficient Δε700 nm of − 50 mM^{-1} cm^{-1}. The ratio PSII/PSI was calculated from the electrochromic shift signals at 50 µs after a single turnover saturating flash (OPO pumped by a pulsed Nd:YAG laser) in treated and non-treated leaves.

Analyses of photosynthetic complexes

Thylakoid preparation was performed by grinding leaves in buffer 1 (0.4 M sorbitol, 0.1 M Tricine-KOH pH 7.5, 10 mM NaCl, 5 mM $MgCl_2$, benzamidine 0.2 mM, aminocaproic acid 1 mM). The solution filtered through a cloth with a pore diameter of 20 µm was centrifuged at 1500×*g* for 15 min at 4 °C. The pellet containing the chloroplasts was resuspended with a brush in buffer 2 (50 mM Sorbitol, 5 mM Tricine pH 7.5, 10 mM EDTA pH 8.0) to explode chloroplasts and then centrifuged again (10,000×*g*, 15 min, 4 °C) to pellet thylakoids. This step was repeated once and thylakoids were finally resuspended in buffer 3 (0.4 M Sorbitol, 50 mM HEPES KOH pH 7.5, 5 mM $MgCl_2$) and stored at − 80 °C.

Protein composition was analyzed by gel electrophoresis and immunoblot. Gels were done using the Laemmli system (Laemmli 1970) with a 14% concentration of acrylamide + bisacrylamide in a ratio 29:1 and 2 M urea. The gels were loaded with 0.78 µg (in tot Chls) of thylakoids per lane. Purified PSII core was used as reference (0.06 µg in Chls) as well purified PSI-LHCI (0.39 µg in Chls). PSII dimeric core was prepared by solubilizing and fractionating Arabidopsis grana membranes on sucrose gradient similarly as in Caffarri et al. (2009), but replacing α-DM (0.3%) with β-DM (1.2% final) for membrane solubilization. The PSII core fraction from the first gradient was loaded on a second gradient to improve purity. PSI-LHCI was purified by elution from a native gel as in (Crepin et al. 2020). Gels were stained with SYPRO Ruby and imaged with the Fusion FX7 revelation system (Vilber). For immunoblot, proteins were transferred on nitrocellulose membranes using the Trans-Blot Turbo Transfer System (Bio-rad), decorated using a home-made CP43 antibody (directed on the *Arabidopsis thaliana* recombinant loop E) and a commercial PsaB antibody (Agrisera) and revealed by chemiluminescence using a Fusion FX7 revelation system (Vilber).

Results

Acclimation of Arabidopsis photosynthesis to light intensity

WT Arabidopsis was grown from seeds at different PFDs ranging from 65 to 750 µmol photons m^{-2} s^{-1}. Figure 1 shows plant growth phenotypes after 5 weeks. Growth was reduced at 65 and 120 µmol photons m^{-2} s^{-1} compared to higher PFDs, and leaf morphology was changed at high PFDs, with an increase in leaf thickness. The leaf specific weight rose from 0.86 mg dry weight (D.W.) cm^{-2} at PFD 65 µmol photons m^{-2} s^{-1} to 1.95 mg cm^{-2} at PFD 750 µmol m^{-2} s^{-1} (Fig. S1A). In parallel, the total chlorophyll content per unit D.W. decreased with increasing PFD during growth (Fig. S1B).

As expected, photosynthetic electron flow capability increased with growth PFD (Fig. 1B). In plants grown at a PFD of 65 µmol m^{-2} s^{-1}, the actual quantum yield of PSII photochemistry (Φ_{PSII}) decreased much more rapidly with increasing PFD compared to plants acclimated to 750 µmol m^{-2} s^{-1}, indicating a faster light saturation of photosynthesis. The light dependence curves of Φ_{PSII} for the other growth conditions [PFDs of 120 and 250 µmol m^{-2} s^{-1} (Fig. 1B) and PFDs of 350 and 550 µmol m^{-2} s^{-1} (not shown)] were intermediate. This acclimation phenomenon typically involves an adjustment of the light-harvesting antenna size of the photosystems which is known to decrease with increasing light intensities (e.g. Ballotari et al. 2007; Dietz 2015; Bielczynski et al. 2016; Mathur et al. 2018). The smaller the light harvesting system the higher the PFD at which photosynthesis is light-saturated. This phenomenon is illustrated here with the changes in the chlorophyll *a*-to-*b* ratio (Fig. 1C). A high ratio indicates a decrease in the chlorophyll *b*-containing antenna system relative to the photosystem reaction centers. This adjustment of the light

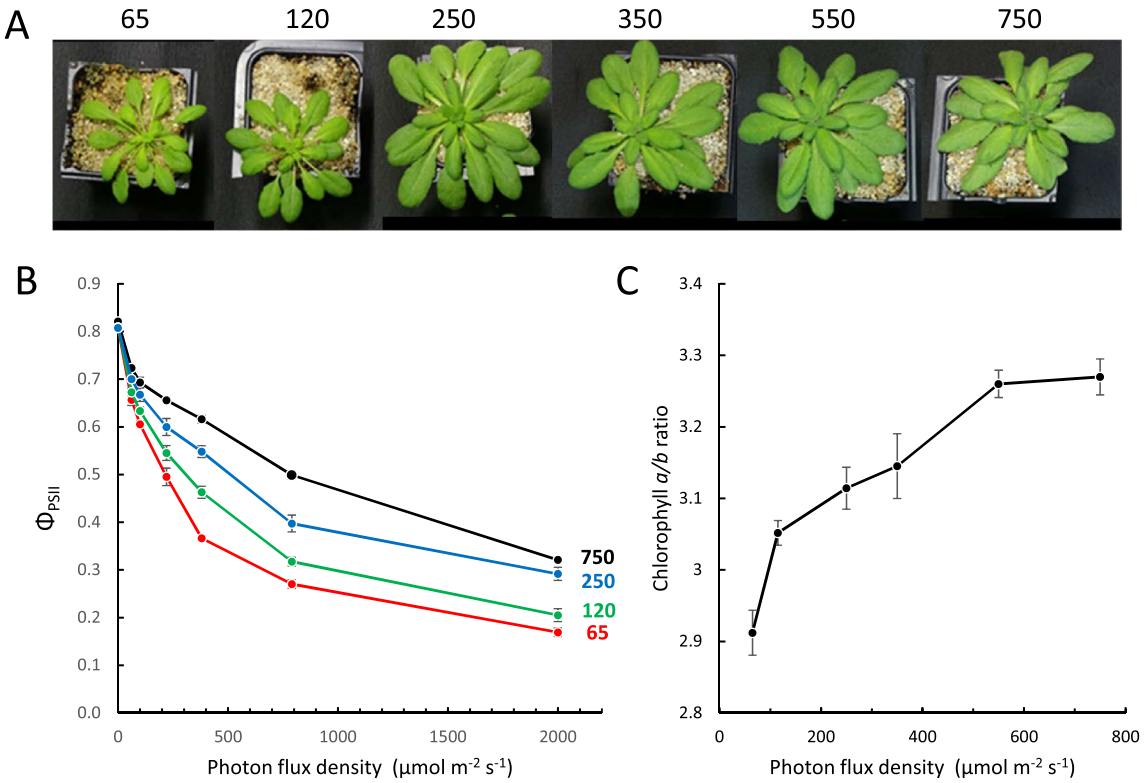

Fig. 1 Growth of WT Arabidopsis at different PFDs. **A** Picture of Arabidopsis plants grown for 5 weeks at different PFDs of white light ranging from 65 to 750 µmol photons m^{-2} s^{-1}. **B** Light dependence of the actual quantum yield of PSII photochemistry (Φ_{PSII}) in Arabidopsis leaves grown at 65, 120, 250 and 750 µmol photons m^{-2} s^{-1}. **C** Chlorophyll *a*/*b* ratio of thylakoids extracted from Arabidopsis leaves grown at different PFDs. Data are mean values of 4 separate mesurements ± SD

harvesting capacities is confirmed below by electrophoresis analyses of the photosynthetic complexes.

PQ accumulation and distribution in plants acclimated to different PFDs during growth

We analyzed the total PQ content (reduced + oxidized PQ) in leaves of plants acclimated to different PFDs. A very marked accumulation of PQ was observed with increasing PFD during growth (Fig. 2A). Compared to plants grown under a PFD of 65 µmol m^{-2} s^{-1}, the PQ content was increased by a factor of 3 in plants grown at 750 µmol m^{-2} s^{-1}. The effect of the light environment was noticeably smaller for α-tocopherol, with a corresponding increase by a factor 1.7. At 750–800 µmol photons m^{-2} s^{-1}, the PQ content was almost five times higher than the tocopherol content. This confirms that PQ is much more responsive to changes in light intensity than other prenyl lipids (Ksas et al. 2015).

The distribution of PQ within the chloroplasts is analyzed in Fig. 2B as a function of growth PFD. Rather surprisingly, the photochemically active PQ pool (i.e. the thylakoid PQ pool involved in photosynthetic electron transport) was virtually independent of the PFD to which plants were acclimated. This pool represented around 0.7 nmol PQ mg^{-1} leaf D.W. at all PFDs. Consequently, the PQ molecules that accumulated in high light-grown plants relative to low light-grown plants (Fig. 2A) were stored in the photochemically non-active pool (Fig. 2B), located mainly in the plastoglobules (Eugeni Piller et al. 2012; Ksas et al. 2018).

Acclimation to high light does not involve substantial changes in the PSII reaction center content

The photosynthetic complexes of Arabidopsis leaves were separated by SYPRO-stained denaturing electrophoresis of thylakoid proteins, using purified PSII core as reference (Fig. 3A). The protein profile of thylakoids as a whole did not change much with increasing PFD during plant growth. The most obvious difference was a decrease in LHCII abundance, as expected. Taking the intensity of the CP43 band (PsbC) as an indicator of PSII core abundance, a slight relative increase in PSII appeared to take place in high light whereas PSI, as indicated by the 'PSI small subunit' band (PSI core complex without Lhca antennae, Crepin et al. 2020), did not seem to be significantly

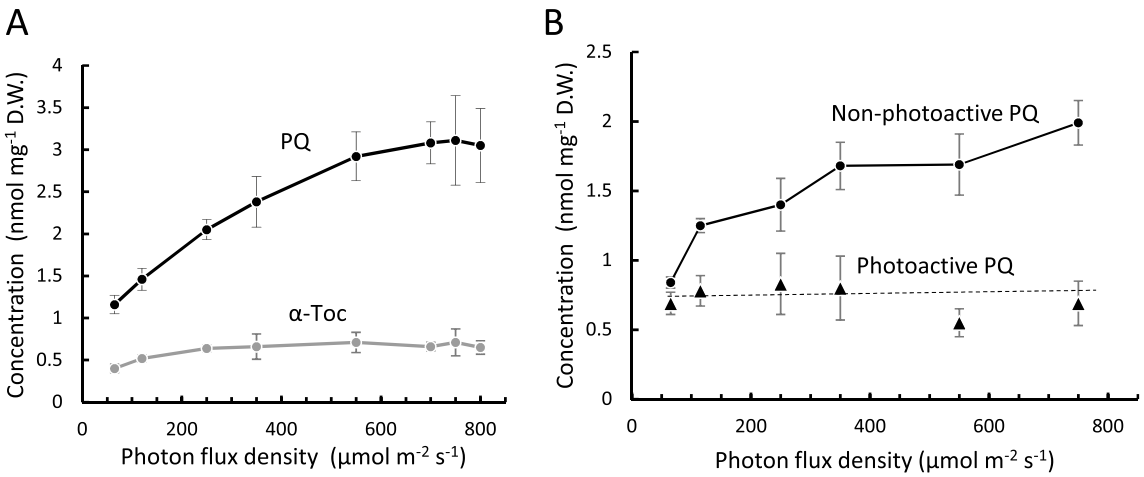

Fig. 2 PQ content and distribution in WT Arabidopsis leaves. **A** Concentration of total PQ and of α-tocopherol in Arabidopsis leaves grown at different PFDs. **B** Size of the photochemically active and inactive pools of PQ in Arabidopsis leaves grown at different PFDs. Data are mean values of 4 separate measurements ± SD

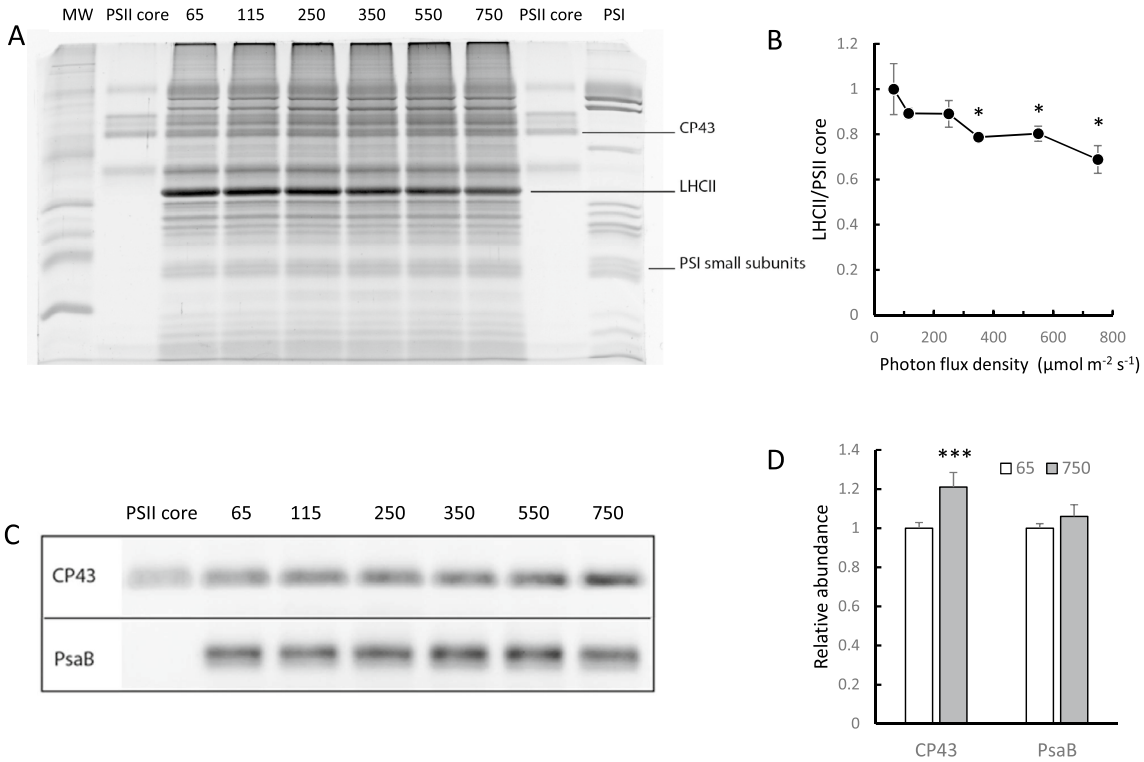

Fig. 3 Photosynthetic complexes in WT Arabidopsis plants grown at different PFDs. **A** SYPRO-stained electrophoresis of thylakoid proteins, purified PSII core and PSI complex. **B** Relative ratio of the LHCII/PSII core calculated from A. **C** Immunoblots of CP43 and PsaB. **D** Relative abundance of CP43 and PSI in low light (65 µmol m^{-2} s^{-1}) vs. high light (750 µmol m^{-2} s^{-1}) estimated from C. Panel **B** and **D** data are mean values of 3 separate measurements + SD. * and ***, different from low light conditions (65 µmol photons m^{-2} s^{-1}) at $P < 0.05$ and $P < 0.001$, respectively (Student's *t*-test)

affected. This is confirmed by immunoblots of CP43 and of the PSI core protein PsaB (Fig. 3C) which show a slight increase in PSII core abundance (CP43) and a stability of PSI level (PsaB) (Fig. 3D) on a chlorophyll basis, the electrophoretic gels being loaded at constant chlorophyll concentration. Considering the chlorophyll concentration

in leaves (Fig. S1), stability of PSI on a chlorophyll basis corresponds to a decrease in PSI on a leaf D.W. basis at high PFDs (about − 30% for the highest PFD). From densitometric evaluations of the PSII core band CP43 and the LHCII band in Fig. 3A, a relative LHCII/PSII core ratio was calculated and normalized to 1 for the 65 µmol photons m^{-2} s^{-1} sample: this ratio exhibited a marked decrease from 1 (at 65 µmol photons m^{-2} s^{-1}) to 0.62 (at 750 µmol photons m^{-2} s^{-1}) (Fig. 3B).

Knowing the amount of chlorophyll *a* loaded on the gels for the purified PSII core, the PSII core content in the samples can be estimated on a chlorophyll basis in % of total chlorophyll from the densitometric intensity of the CP43 band in comparison to that of the purified PSII core. The PSII core was found to represent ~ 9% of total chlorophyll in low light (65 µmol photons m^{-2} s^{-1}) and almost 13% in high light (750 µmol m^{-2} s^{-1}) (Fig. 4A). Based on the chlorophyll content of leaves grown at different PFDs (Fig. S1), we were able to express the PSII core content in chlorophyll *a* per mg leaf D.W. (Fig. 4B). The PSII core content was found to be almost independent of the growth PFD, showing only a slight decrease with increasing PFD.

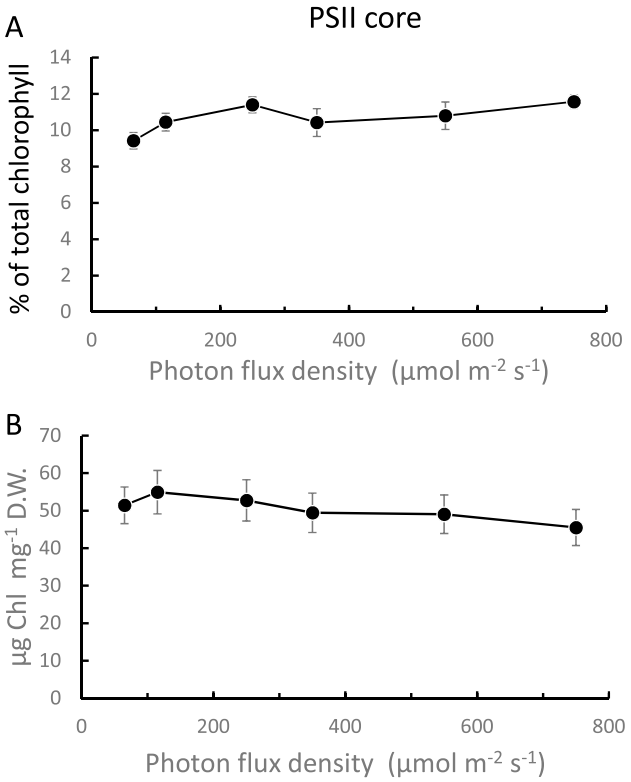

Fig. 4 PSII core content in WT Arabidopsis leaves grown at different PFDs. **A** Chlorophyll *a* in PSII core in % of total chlorophyll. **B** PSII core concentration in leaves in chlorophyll *a* per leaf D.W. Data are mean values of 3 measurements ± SD

A quantitative determination of the PSI and PSII reaction center pigments in leaves was performed using spectroscopic methods. P700 absorbance spectroscopy measurements showed that the PSI reaction center concentration (on a leaf D.W. basis) decreased with increasing PFD during growth (Fig. 5A). The PSII-to-PSI ratio, estimated from flash-induced electrochromic shift measurements, was just below 1 in low light conditions (as expected, Wientjes et al. 2017) and increased with light intensity (Fig. 5A). Those changes are in line with previous reports on the acclimation of Arabidopsis to the light environment (Bailey et al. 2001; Ballottari et al. 2007). From those two parameters, the PSII content can be calculated (Fig. 5B). Similarly to photoactive PQ and in agreement with the biochemical analyses, the PSII reaction center (P680) concentration exhibited

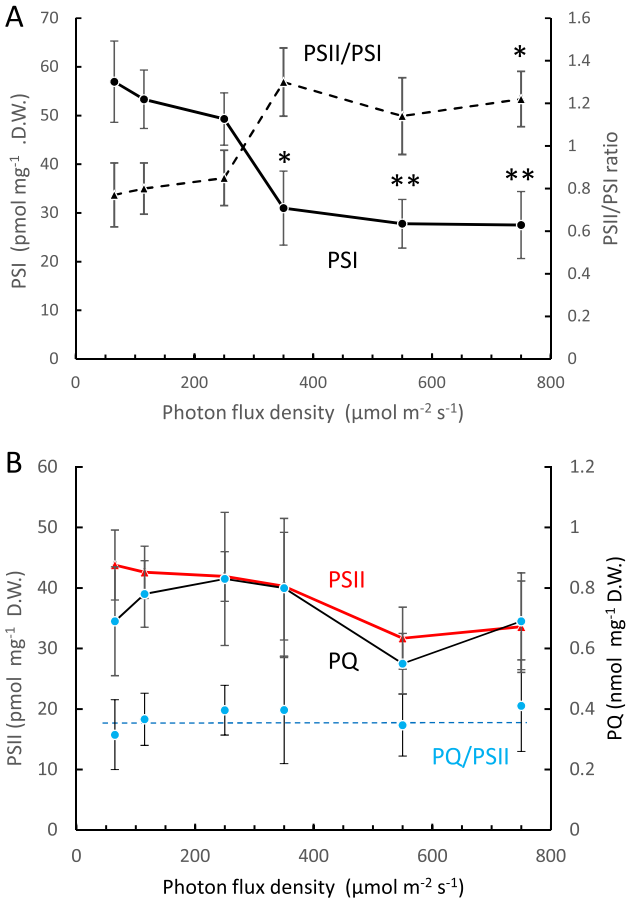

Fig. 5 PQ-to-PSII ratio in WT Arabidopsis leaves grown at different PFDs. **A** PSI reaction center pigment and PSII/PSI ratio as determined by P700 absorption spectroscopy and flash-induced electrochromic shift. **B** PQ content (black symbols and lines), PSII content (red symbols and lines) and PQ/PSII ratio (blue symbols and dotted line) in Arabidopsis leaves. The concentration of PSII reaction centers was calculated from A. Data are mean values of 3 repetitions ± SD. * and **, significantly different from low light conditions (65 µmol photons m^{-2} s^{-1}) at $P < 0.05$ and $P < 0.01$, respectively (Student's *t*-test)

relatively little changes with the modification of PFD during plant growth. The PSII reaction center content was about 30–40 pmol mg^{-1} D.W. Because the PQ and PSII profiles with PFD were very similar, the PQ-to-PSII ratio was virtually constant in the plants grown at different PFDs, with an average value of 17 PQ molecules per PSII.

Homeostasis of the photochemically active PQ pool

Overexpression of the PQ biosynthesis gene *SPS1* (*SOLANESYL DIPHOSPHATE SYNTHASE 1*) in Arabidopsis leads to a stimulation of PQ synthesis and an enhanced PQ content in leaves (Ksas et al. 2015). This is confirmed here in two *OE:SPS1* lines which exhibited a strong accumulation of total PQ compared to WT, particularly at high PFDs (Fig. 6A). At low PFDs (65–120 µmol m^{-2} s^{-1}), the PQ content in *OE:SPS1* leaves was increased by 50%, as previously reported (Ksas et al. 2015). At high PFDs (700–800 µmol m^{-2} s^{-1}), the rise in PQ levels in *OE:SPS1* relative to WT was more than twofold. This strong increase in PQ appears to have a negative impact on tocopherol synthesis, presumably by competition of the two biosynthesis pathways for the common precursor homogentisic acid (Havaux 2020).

PQ accumulation in the *OE:SPS1* lines had no effect on the amounts of photoactive PQ which remain similar in WT and *OE:SPS1* leaves at all light intensities (Fig. 6B). Therefore, the extra PQ in the *SPS1* overexpressors was stored in the non-photoactive PQ pool. This constant PQ levels in the thylakoid membranes of WT and *OE:SPS1* was associated with similar efficiencies of photosynthetic electron transport (Fig. 7).

Reduced size of the photoactive PQ pool and photosynthetic efficiency

Dynamic exchange of metabolites is believed to take place between the plastoglobules and the thylakoid membranes (Austin et al. 2006a, b; Bréhélin and Kessler 2008; Pralon et al. 2020). The plastoglobular kinase ABC1K1 has been identified as a possible player in this transfer of biomolecules, especially PQ, from the plastoglobules to the thylakoid membrane (Pralon et al. 2019). Mutational suppression of ABC1K1 in the *abc1k1* knockout mutant was shown to inhibit the delivery of PQ from the plastoglobules to the thylakoid membranes (Pralon et al. 2019). As shown in Fig. 8A, the total amounts of PQ in the *abc1k1* mutant were not significantly different from the WT amounts at all PFDs. However, the concentration of thylakoid photoactive PQ molecules was markedly reduced in the mutant to about 50% of WT levels in the PFD range 65–550 µmol photons m^{-2} s^{-1}, with values close to 0.4 nmol mg^{-1} D.W. (Fig. 8B). The lower PQ levels in the thylakoid membranes of *abc1k1* were accompanied by a corresponding increase in the non-photoactive PQ levels. However, at the highest PFDs (550–750 µmol m^{-2} s^{-1}), the photoactive PQ pool in

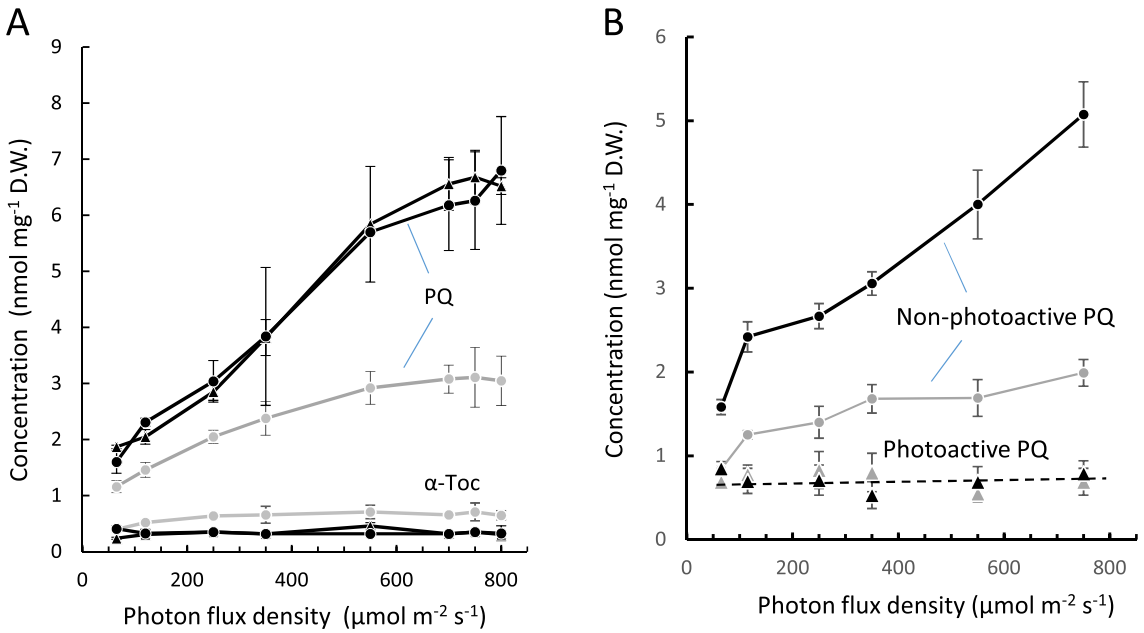

Fig. 6 PQ in Arabidopsis *OE:SPS1* leaves grown at different PFDs. **A** Total PQ content and α-tocopherol content in leaves of WT Arabidopsis and two transgenic lines (L3 and L12) overexpressing the *SPS1* gene (*OE:SPS1*). WT, grey symbols and lines; *OE:SPS1* L3, black triangles; *OE:SPS1* L12, black circles. **B** Photochemically active and inactive PQ pools in WT and *OE:SPS1* plants grown at different PFDs. WT, in grey; *OE:SPS1* L12, in black. Data are mean values of 4 measurements ± SD

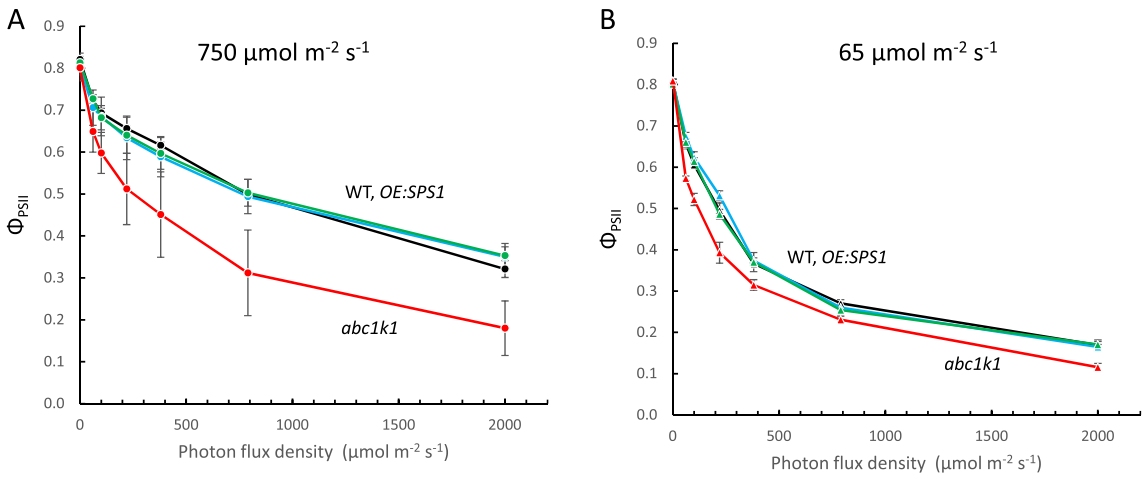

Fig. 7 Photosynthetic electron flow in Arabidopsis leaves grown at different PFDs. Light dependence of the actual quantum yield of PSII photochemistry (Φ_{PSII}) in leaves of WT Arabidopsis, *OE:SPS1* transgenic lines (L12, L3) and *abc1k1* mutant grown at 65 or 750 µmol m^{-2} s^{-1}. *abc1k1*, red; WT, black; *OE:SPS1* L3, green; *OE:SPS1* L12, blue. Data are mean values of 4 repetitions ± SD

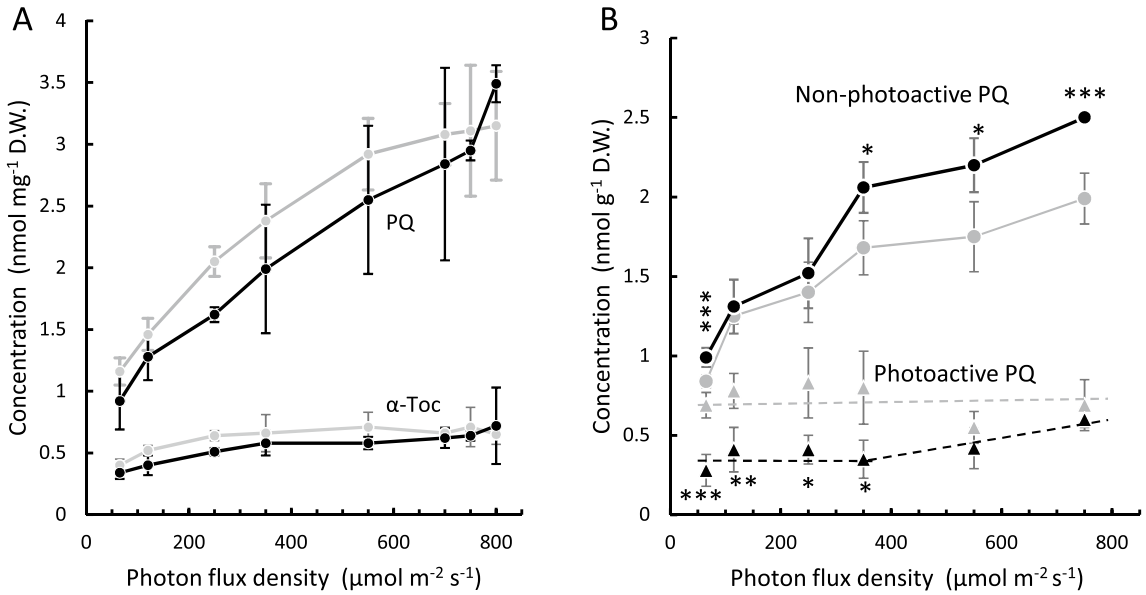

Fig. 8 PQ in the Arabidopsis *abc1k1* mutant grown at different PFDs. **A** Concentration of total PQ and of α-tocopherol in leaves of WT Arabidopsis and *abc1k1* mutant. **B** Size of the photochemically active and inactive pools of PQ in leaves of WT Arabidopsis and *abc1k1* mutant. WT, in grey; *abc1k1*, in black. Data are mean values of 4 or 5 repetitions ± SD. *, ** and ***, significantly different from WT at $P < 0.01$, 0.05 and 0.001, respectively (Student's *t*-test)

abc1k1 leaves tended to increase and was not significantly different from the WT pool. Thus, it seems that strong accumulations of PQ in high light can somehow overcome the lack of ABC1K1 kinase for PQ delivery to the thylakoid membranes. The regulation of metabolite exchange between thylakoid membranes and plastoglobules as well as the exact role of ABC1K1 in this process remain to be elucidated.

As previously reported, the photosynthetic electron flow efficiency was decreased in *abc1k1* compared to WT (Fig. 7). This was observed at all growth PFDs, but the effect was more pronounced in plants grown at the highest PFDs, although the difference in the photoactive PQ pool size between WT and *abc1k1* was attenuated in high light (Fig. 8). This indicates that other factors impacted electron transport in *acb1k1* leaves acclimated to high PFDs.

Discussion

PQ accumulates in high light in a non-photoactive state

Higher concentrations of PQ in high light-grown leaves compared to shade-acclimated leaves was previously reported in several plant species (Lichtenthaler 2007; Szymanska and Kruk 2010). This study confirms that PQ is highly responsive to the light environment, with the PQ content of leaves markedly rising with increasing light intensity. This response is exacerbated compared to other prenyl lipids such as tocopherol (Fig. 2A) or plastochromanol (Ksas et al. 2015). However, the accumulation of PQ in high light is essentially constituted by non-photoreducible PQ molecules. They are stored outside the thylakoid membranes, in the plastoglobules and in the chloroplast envelopes (Ksas et al. 2018). This pool was found to represent from 50% (in low light) up to 75% (in high light) of total PQ. Similarly elevated values were previously reported in Arabidopsis and barley leaves (Pshybytko et al. 2008; Szymanska and Kruk 2010). Actually, the capacities of PQ storage are very high: in *OE:SPS1* leaves grown at 800 µmol photons m^{-2} s^{-1}, boosting PQ biosynthesis led to a large non-photoactive PQ pool that represented almost 95% of total PQ (Fig. 6B). Thus, plastoglobules, and possibly chloroplast envelopes, are major storage sites allowing accumulation of the extra PQ molecules that are in excess relative to the requirements of photosynthesis. This is in line with the marked increases in the size and number of plastoglobule lipid droplets previously reported in high light-exposed plants (Lundquist et al. 2013; Van Wijk and Kessler 2017; Zechmann 2019; Espinoza-Corral et al. 2021). Since the non-photoactive PQ molecules are not directly connected to the photosynthetic electron flow, they likely fulfill functions different from electron shuttling in photosynthesis. A number of non-photochemical functions have been shown for PQ including co-factor in biosynthesis reactions, chloroplast signaling and antioxidant activities (Havaux 2020). The strong accumulations observed during acclimation to high light would fit with a photoprotective function. This can be related to the capacity of PQ to scavenge $^1O_2$ (Yadav et al. 2010), generating various oxidation products including hydroxy- and trihydroxy-PQ (Ferretti et al. 2018). It has been proposed that the plastoglobular PQ fraction is a reservoir to re-fill the thylakoid pool after PQ oxidation by $^1O_2$ produced by PSII (Ksas et al. 2018). Accordingly, the large pool of non-photoactive PQ in *OE:SPS1* was associated with increased phototolerance (Ksas et al. 2015). Conversely, impairment of PQ transfer from the plastoglobules to the thylakoid membranes in *abc1k1* led to a photosensitive phenotype (Pralon et al. 2019; Lundquist et al. 2013; Martinis et al. 2014). As confirmed in Fig. S2, growth of *abc1k1* plants in high light was reduced compared to WT and *OE:SPS1* plants while they were undistinguishable in low light.

The photoreducible PQ pool size and the PSII reaction center content in Arabidopsis leaves show little sensitivity to PFD levels during plant growth

The most striking result of this study is the constant concentration of thylakoid photoreducible PQ in leaves. The amount of photochemically active PQ was virtually similar in WT Arabidopsis grown at 65 and at 750 µmol photons m^{-2} s^{-1} while the total amounts were very different. Even the strong accumulation of PQ in high light-grown *OE:SPS1* plants did not change the photoactive PQ levels. This pool was measured here by direct quantification of reduced and oxidized PQ molecules and not by functional analyses (*e.g.* chlorophyll fluorescence), and full reduction of PQ was achieved by illumination with intense, saturating white light. The results are therefore not influenced by changes in PQ functions such as non-photochemical energy quenching (Haldimann and Tsimilli-Michael 2005). Moreover, a validation of our method was provided by Ksas et al. (2018) who previously showed that the PQ amounts measured in thylakoid membranes and plastoglobules after chloroplast fractionation are comparable with the PQ amounts in the photoactive/non-photoactive pools as measured in this study. The results obtained with the two methods were similar even under conditions associated with PQ accumulation in the leaves.

Because the amounts of PSII reaction center were not significantly modulated during photoacclimation (Fig. 3), the PQ/PSII level is another parameter that does not seem to change much during acclimation to high light. The stability of PSII reaction center proteins in high light-acclimated compared to low light-acclimated leaves was also reported in a recent proteomic study (Fannery et al. 2021). In contrast, increase in PSII content in high light-acclimated Arabidopsis plants was reported in earlier studies when expressed per unit chlorophyll (Bailey et al. 2001). Since the chlorophyll concentration in Arabidopsis leaves was found to substantially decrease as PFD increased (Fig. S1B), this increase could actually be absent, or at least attenuated, when PSII content is expressed on a leaf mass basis. Similarly to our results (Fig. 5A), Ballottari et al. (2007) also found an increase of about 20% in the PSII/PSI ratio in Arabidopsis plants acclimated to high light (1600 µmol photons m^{-2} s^{-1}) compared to low light-grown plants (25–100 µmol m^{-2} s^{-1}).

From the quantification of PSII reaction center and PQ levels, the PQ/PSII ratio was calculated to be about 17 PQ per PSII at all PFDs. This value is in the upper range of previous estimates of PQ/PSII (5–20) in various plant materials

by biophysical methods involving fluorescence or absorbance spectroscopy (Forbush and Kok 1968; Stiehl and Witt 1969; Ksas et al. 2018; McCauley and Melis 1986). This value could be species-dependent, but it could also be influenced by environmental factors different from light intensity (Pshybytko et al. 2008). Our finding that the concentration of photoactive PQ is independent of the PFD at which Arabidopsis is acclimated does not support the idea that efficient photosynthesis of shade-grown plants involves enhancement of the intersystem PQ pool size to facilitate electron transport in limiting light conditions (Suslichenko and Tikhonov 2019). Adjustment of the PSII light-harvesting antenna size appears to be a more crucial mechanism in photoacclimation (Fig. 3).

Photoreducible PQ pool size and photosynthetic efficiency

A constant, PFD-independent PQ/PSII ratio in Arabidopsis leaves could suggest that a set number of PQ molecules is necessary for efficient interaction with PSII and optimal photosynthesis. This can be related to the dense packing of thylakoid membranes with various protein complexes which is likely to limit long-range diffusion of mobile PQ within the membrane lipid phase (Kirchhoff et al. 2002). The current view of the photosynthetic electron transport machinery favors an organization in supercomplexes, with a close compartmentalization of PSII, PQ, and the cytochrome *b6/f* complex in membrane microdomains (Kirchhoff et al. 2000; Johnson et al. 2014). These microdomains may accommodate a limited number of PQ molecules involved in rapid exchange between PSII and cytochrome *b6/f*. As a corollary, manipulation of the PQ biosynthesis pathway to enhance PQ levels (as in *OE:SPS1*) does not appear to be a promising way to enhance photosynthetic efficiency since the extra PQ will be stored in the plastoglobules and/or in the chloroplast envelopes. However, as previous shown (Ksas et al. 2015; Ferretti et al. 2018), this does not exclude that PQ accumulation in leaves is advantageous against stress conditions. Constitution of a large pool of non-photoactive PQ can enhance the antioxidant capacities and hence the tolerance to photooxidative stress.

The average concentration of photoactive PQ in Arabidopsis leaves was around 700 pmol mg^{-1} D.W. (Fig. 2B). A deviation towards lower values, as in the *abc1k1* leaves (*ca.* 400 pmol mg^{-1} leaf D.W.), was associated with decreased efficiency of photosynthetic electron transport. This observation would support the idea that the PQ levels in the thylakoid membranes are optimized to ensure maximal photochemical efficiency. However, the effect of the *abc1k1* mutation on the photoactive PQ pool was noticeably attenuated in high light, and this was associated with a stronger difference in Φ_{PSII} between WT and *abc1k1*. There was also a marked difference in growth phenotype between the two genotypes at 750 µmol photons m^{-2} s^{-1} (Fig. S2) indicating that other factors from the size of the PQ pools per se are involved in the reduced development and photosynthetic efficiency of *abc1k1* plants in high light. In fact, neither the in vivo target nor the regulation of ABC1K1 kinase are known. ABC1K1 could phosphorylate several targets, leading to a complex regulation of photosynthetic activities which remains to be elucidated. Accordingly, acclimation to high light was shown to result in targeted changes in the protein and prenyl lipid composition of plastoglobules which were perturbed in the Arabidopsis *abc1k1 abc1k3* double mutant (Espinoza-Corral et al. 2021).

Conclusions

The maintenance of a given size of the PQ pool in the thylakoid membranes requires a tight regulation of PQ distribution between the different chloroplast membranes. Based on the physical continuity between the plastoglobules and the thylakoid membranes, it has been postulated that dynamic exchanges of metabolites exist between them. For instance, the last step of tocopherol biosynthesis occurs in the plastoglobules (Vidi et al. 2006), implying a transfer mechanism of the newly synthesized tocopherol molecules to the thylakoid membranes. Since oxidation of PQ occurs in the thylakoid membranes where ROS are produced in the light (Ksas et al. 2018), new PQ molecules must be continuously delivered from the plastoglobules to replace photooxidized PQs. Transfer of Arabidopsis plants from low light to high light was previously shown to be associated with the accumulation of PQ oxidation products in the thylakoid membranes (Ksas et al. 2018). Although the molecular mechanisms underlying this exchange are still unknown, a number of plastoglobular proteins have recently been implicated in this exchange. The kinase ABC1K1 was found to favor PQ transfer from the plastoglobules to the thylakoid membranes (Pralon et al. 2019). This was confirmed here, at least for PFDs < 550 µmol m^{-2} s^{-1}, by the decrease in the photoreducible PQ pool in *abc1k1* leaves (compared to WT) in favor of the non-photoreducible pool. Another kinase, ABC1K3, would act in an opposite manner, limiting this transfer (Pralon et al. 2020). Moreover, the different steps of PQ biosynthesis occur in different compartments of the chloroplast, also requiring tight cooperation and complex transport systems. The dynamics of PQ partitioning in the chloroplast as well as the molecular targets of ABC1K1 and ABC1K3 are still elusive and will be a major challenge in future research on PQ metabolism and functions. Hopefully, future works will identify the mechanism by which the optimal size of the photoactive PQ pool is maintained in the chloroplasts.

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Author contributions MH conceived the project and designed the experiments. BK performed most experiments. JA performed biophysical analyses of PSII content. SC performed biochemical analyses of the photosynthetic complexes. MH wrote the article with input from all the other authors.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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