PSB33 protein sustains Photosystem II in plant chloroplasts under UVA light

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Highlight
Plants utilize light for growth. As light differentiates during a day, month or year, it serves as a trigger for acclimation. Here we show that PSB33 is involved in a UVA light-mediated mechanism to sustain Photosystem II.
Abstract
Plants can quickly and dynamically respond to spectral and intensity variations of the incident light. These responses include activation of developmental processes, morphological changes, and photosynthetic acclimation that ensure optimal energy conversion and minimal photoinhibition. Plant adaptation and acclimation to environmental changes have been extensively studied, but many details surrounding these processes remain elusive. The Photosystem II (PSII) associated protein PSB33 plays a fundamental role in sustaining PSII as well as in the regulation of the light antenna in fluctuating lights. We investigated how PSB33 knock-out plants perform under different light qualities. psb33 plants displayed 88% lower fresh weight compared to wild type plants when cultivated in the border of UVA-blue light. The sensitivity towards UVA light was associated with a lower abundance of PSII proteins, which reduces psb33 plants’ capacity for photosynthesis. The UVA phenotype was further found to be linked to altered phytohormone status and changed thylakoid ultrastructure. Our results collectively show that PSB33 is involved in a UVA light-mediated mechanism to maintain a functional PSII pool in the chloroplast.

Key words
Arabidopsis, Blue light, Photoinhibition, State transition, Thylakoid membrane, UV light.
Introduction
The absorbed light energy from the sun is not always beneficial for plants - in excess or under periods of environmental stress, the light causes inhibition of photosystem II (PSII) activity that may result in damage to the photosynthetic apparatus, a phenomenon referred to as photoinhibition (Kok, 1956; Li et al., 2018). To minimize photoinhibition, plants have evolved protective mechanisms that ensure functional photosynthesis also under light stress conditions (Long et al., 1994; Sarvikas et al., 2006; Scheller and Haldrup, 2005; Yamamoto, 2016). These responses include limiting and dissipation of excess of absorbed light energy, and damage control by scavenging reactive oxygen species (ROS).

The thylakoid PSII auxiliary protein PHOTOSYSTEM II PROTEIN 33 (PSB33, AT1G71500) plays a specific role in the regulation of photoinhibition. PSB33 influences PSII-LHCII supercomplex organization in Arabidopsis (Fristedt et al., 2015), and plants devoid of PSB33 display reduced state transition, i.e., ability to distribute excitation energy between the two photosystems (Fristedt et al., 2017). Furthermore, psb33 mutants show stunted growth in moderate, high, and fluctuating lights (Fristedt et al., 2015; Fristedt et al., 2017).

When we started cultivating psb33 mutant plants in our new growth facility, we were unable to reproduce our previous finding that the mutant grows slower than wild type under standard conditions (Fristedt et al., 2015). Instead of the expected 50% reduction in fresh weight compared to wild type, psb33 mutants grew to almost similar size (Figure S1). We hypothesized that this variability in the size of psb33 attributes to light spectral differences since the light intensity was identical between these two experiments.

Photoinhibition in plants is initiated by photoinactivation of PSII and followed by a repair cycle of the damaged subunits. There is currently no consensus regarding how photoinactivation of PSII is initiated and sensed by the plant. The two leading hypotheses, which are not mutually exclusive, are I) the acceptor-side PSII photoinhibition mechanism induced by singlet oxygen in excessive light, and II) the donor-side PSII photoinhibition occurring via light absorbed by the Mn₄O₅Ca cluster of PSII and occurring even in very low light intensities (Aro et al., 1993; Zavafer et al., 2015). However, from studies on the action spectrum of PSII photoinactivation, it is evident that the extent of photoinhibition is wavelength dependent and that light in the UV region of the spectrum (100-400 nm) causes
the strongest photoinactivation of PSII (Jones and Kok, 1966; Sarvikas et al., 2006; Takahashi et al., 2010), predominantly targeting the Mn$_4$O$_5$Ca cluster of the reaction center and the acceptor side of PSII (Hakala et al., 2005; Ohnishi et al., 2005). Thus, non-photochemistry wavelengths can substantially affect PSII photoinhibition.

The relative excitation pressure on the two photosystems, PSI and PSII, changes depending on light intensity and spectral properties, and the inability to appropriately distribute the light energy between the photosystems may lead to photoinhibition (Bellafiore et al., 2005). Plants can reallocate a fraction of the light-harvesting antenna complex II (LHCII) between PSI and PSII through a process known as state transition (Adamska et al., 1992; Allen et al., 1981). The phosphorylation of the LHCII antenna proteins turns on this reallocation (Mekala et al., 2015). High excitation pressure on PSII activates the serine-threonine kinase STN7 that phosphorylates LHCII proteins (Bellafiore et al., 2005; Fristedt and Vener, 2011; Trotta et al., 2016). This results in enhanced delivery of excitation energy from LHCII to PSI, thus increasing its light-harvesting capacity (State II). Over-excitation of PSI, on the other hand, triggers the dephosphorylation of LHCII by the PPH1/TAP38 phosphatase (Pribil et al., 2010; Shapiguzov et al., 2010). Dephosphorylated LHCII associates with PSII to increase its capacity to absorb energy (State I). Under artificial illumination, State I is induced by low-intensity light (Rintamäki et al., 2000) as well as red (636-700nm) and blue (400-500 nm) light (Bellafiore et al., 2005; Trotta et al., 2016). Far-red light (700-780 nm) preferentially excites PSI and triggers State II (Adamska et al., 1992; Bonardi et al., 2005).

Repair of photodamaged PSII proteins is efficient and under strict quality control, especially of the D1 subunit. This photoinhibition/repair cycle follows a sequential series of highly regulated steps: monomerization of damaged PSII dimers, migration of PSII monomers from appressed to unappressed thylakoid regions, degradation of the damaged D1 protein by FtsH protease, and finally, de novo production of D1 subunits insertion into PSII monomers (Ohad et al., 1984; Yoshioka-Nishimura and Yamamoto, 2014). Phosphorylation of the PSII core proteins by the STN8 kinase is important for the repair cycle and influences the disassembly of the damaged PSII dimer and protects D1 protein from excess degradation (Fristedt et al., 2009; Kato and Sakamoto, 2014; Tikkanen et al., 2008).
Apart from directly fueling photochemistry, light of different colors and intensities serves as cues during plant development and growth. A large number of photoreceptors allow plants to sense light over a broad spectrum of wavelengths, from UV-B (280-320 nm) to far-red. Phytochromes absorb red and far-red light in the 600-750 nm region and play central roles in plant germination, de-etiolation, stomata development, flowering, shade avoidance, and senescence (Wang and Wang, 2014). Blue light and UV light are perceived and effectively transduced as a signal by the photoreceptor families cryptochromes (400-500 nm), phototropins (320-500 nm), zeitlupes (450-520 nm) and UV-B photoreceptors (280-320 nm) (Christie et al., 2015). These photoreceptors control processes that include photomorphogenesis, flowering, circadian period, phototropism, and stomata opening. Signaling pathways downstream of the photoreceptors often integrate with hormone networks and influence their levels (Chaiwanon et al., 2016). Although well studied, there is limited evidence linking photoreceptors directly to photosynthesis regulation. Photoprotective mechanisms identified in green algae and cyanobacteria have been tied to UV and or blue light perception by photoreceptors (Allorent et al., 2016; Kirilovsky and Kerfeld, 2013; Petroutsos et al., 2016). Further, blue light perception in diatoms increases their photoprotective potential (Schellenberger Costa et al., 2013). However, these are aquatic organisms exposed to an environment where blue light dominates. In land plants, phototropins activated by blue light stimulate the relocation of chloroplast (Goh, 2009). This process acts to distribute PSII damage among the different cell layers of the leaf in high light (Cazzaniga et al., 2013). More indirectly, blue and UVA (320-400 nm) light play a role in the regulation of the PSII complex as they stimulate the expression of genes encoding Light-harvesting chlorophyll a/b-binding proteins (Lhcb3) (Folta and Kaufman, 1999) and a special class of PSII core proteins (PSBD-PSBC, D2, and CP43) (Christopher and Mullet, 1994).

Here, we aimed to investigate if changes in light quality affect growth and photosynthetic responses in psb33 mutant plants. We found that the psb33 plants show a conditional dwarf phenotype when cultivated under blue light, specifically in UVA or near UVA light. This UVA light-dependent phenotype was found to be associated with low levels of PSII proteins, abnormal thylakoid ultrastructure, and changed phytohormone levels. We suggest that PSB33 is involved in a UV light-mediated mechanism to maintain an efficient PSII repair by regulating the de novo synthesis of PSII.
Material and Methods

Plant material and growth conditions

*Arabidopsis thaliana* seeds were sown on soil and vernalized at 4°C for two days before transfer to climate-controlled growth chambers (CLF PlantMaster, Plant Climatics, Wertingen, Germany). The plants were grown under a 12h photoperiod at 20°C/18°C light/dark in 60% relative humidity. Seedlings were transplanted into individual pots after ten days and moved to chambers with LED lamps after an additional four days. All mutants and wild type used in the study were in the Columbia-0 genetic background, previously described for *psb33-3* (Fristedt et al., 2015), *stn7* (Bellafiore et al., 2005), *stn8* and *stn7stn8* (Bonardi et al., 2005), *tap38* (Pribil et al., 2010), *amiLhcb1* and *amiLhcb2* (Pietrzykowska et al., 2014), *psbw* (García-Cerdán et al., 2011), *psal* (Lunde et al., 2000), *pgr5* (Munekage et al., 2002) and *npq4* (Johnson and Ruban, 2010). The LED lights were provided by Heliospectra L4A lamps (Heliospectra, Gothenburg, Sweden) with diode peak wavelengths at 400, 420, 450, 530, 630, 660, and 735 nm. For the experiment with the 385 nm LEDs, Heliospectra RX30 lamps (Heliospectra, Gothenburg, Sweden) were used. Full light spectra were recorded with a JAZ spectrometer (Ocean optics, Dunedin, FL, USA). Lamps were set up to ensure an even light distribution of the different colors. For example, when blue and red light were combined, 50% of the total photon flux was provided by blue LEDs and 50% by red LEDs.

Pigment content, hormonal analysis, and chlorophyll a fluorescence

Chlorophyll and carotenoids were extracted from leaf discs in 95% (v/v) ethanol for 10 min at 60°C and concentrations were determined spectrophotometrically (Lichtenthaler and Wellburn, 1983). Plant hormone levels were determined from leaf discs (12 mm diameter) as described (Floková et al., 2014).

Chlorophyll a fluorescence parameters were recorded with a Pocket PEA Chlorophyll Fluorimeter (Hansatech Instruments Ltd, King’s Lynn, Norfolk, UK). The maximum quantum efficiency of Photosystem II (Fv/Fm, calculated as (Fm-F0)/Fm) and performance index (Pl_{ABS}) (Strasser et al., 2004) were determined in attached leaves dark-acclimated for 20 min in room temperature with 1 s measuring light with the intensity of 3500 µmol s^{-1}. 
**Thylakoid membrane preparation, SDS-PAGE and immunoblotting**

For extraction of whole leaf proteins, tissue (approximately 200 mg) was frozen in liquid nitrogen, grounded into a fine powder, mixed with buffer (100 mM Tris-HCl pH 8.0, 25 mM EDTA pH 8.0, 250 mM NaCl, 0.75% SDS (v/v), 1 mM DTT, 10 mM NaF, 1X Protease inhibitor cocktail set I (Calbiochem, Merck KGaA, Darmstadt, Germany)) and denatured at 68°C for 10 minutes. Centrifugation removed cellular debris, and chlorophyll content was determined as described (Porra et al., 1989). Proteins were loaded on 5%/14% stacking/separating acrylamide gels based on chlorophyll content (100-500 ng µl-1 depending on the expected abundance of the protein). SDS-PAGE separation, protein transfer to PDVF membranes, and detection were as described (Fristedt et al., 2015). Antibodies used in this work were all from Agrisera Antibodies (Vännäs, Sweden): PsaB, PsaA, RbscL, PsbA, PsbD, Lhcb2-P (AS13 2705), Lhcb2 (AS01 003), Lhcb1-P (AS13 2704), Lhcb1 (AS01 004).

**Staining for reactive oxygen species**

Staining for H$_2$O$_2$ was performed as previously described with minor modifications (Daudi and O’Brien, 2012). Briefly, seedlings were vacuum inoculated in 10 mM Na$_2$HPO$_4$ 3,3’-diaminobenzidine (Saadatian-Elahi et al.) staining solution at approximately 100 mbar for 2 min. After two hours in darkness, the seedlings were destained in 96% (v/v) ethanol overnight and photographed. Staining for superoxide radicals was performed similarly but using 0.1% nitroblue tetrazolium in 50 mm Na$_2$HPO$_4$ (pH ~7).

**Transmission electron microscopy**

Leaves from plants grown in fluorescent white, red, or blue light were collected two hours after the onset of light following a 14h night period. Tissue preparation and imaging were as described (Herdean et al., 2016).

**PSII electron transfer properties**

Electron transfer properties in PSII were measured by flash-induced variable fluorescence, thermoluminescence, and low-temperature electron paramagnetic resonance spectroscopy (EPR) in thylakoid membranes isolated from wild type and psb33 mutant plants. Fluorescence decay kinetics were measured using an FL3000 dual modulation kinetic fluorometer, and thermoluminescence signal was measured using a TL200/PMT.
A thermoluminescence system (both from Photon System Instruments, Brno, Czech Republic) as described in (Volgusheva et al., 2016).

Low-temperature EPR was measured in X-band ELEXSYS 500 spectrometer using a SuperX EPR049 microwave bridge and a Bruker 4122SHQE super high-Q cavity (Bruker BioSpin). The system was fitted with a 900-cryostat and an ITC-503 temperature controller from Oxford instruments Ltd. The S₂ state multiline signal was induced by illumination at 200 K for 6 min, and full oxidation of Cytochrome b₅₅₉ was induced by illumination at 77 K as described in (Chen et al., 2011). Processing and analysis of EPR spectra were performed in Bruker Xepr program.

Statistical analyses
Data was handled in Microsoft Excel 2010 (Microsoft, USA) with the add-in Daniel’s XL Toolbox (https://www.xltoolbox.net/) or in IBM SPSS Statistics version 25 (IBM corporation, Armonk, US). The normality of data was tested using the Shapiro-Wilk method. Non-normally distributed data were log-transformed before parametric tests were performed. Results were considered significant if two-tailed \(p<0.05\).

Results
psb33 mutant plants show a conditional light quality phenotype
We set out to investigate if the lack of a slow-growth phenotype of psb33 in our new plant growth facility might be attributed to specific qualitative properties of the fluorescent lights in these chambers (Figure S1). Therefore, plants were grown under fluorescent white light at a total photon flux density of 120 \(\mu\text{mol photons m}^{-2}\text{s}^{-1}\) (PFD) for two weeks and then transferred to blue [400,420,440] nm, red [630,660] nm, or a combination of blue and red LED lights, for additional three weeks (Figure 1). Wild type and control plants were maintained in the fluorescent white light throughout the growth period. The total light intensity was kept constant at 120 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) in all different spectral conditions (Figure 1, left panel). To test if the growth phenotype of psb33 was associated with its inability to induce state transition, the double mutant stn7stn8 was included as a negative control in the experiment (Tikkanen et al., 2010). After two weeks of growth under fluorescent white
light, before transfer to LED lights, there was no apparent difference in size between the wildtype, psb33 and stn7stn8 (Figure S2).

The stn7stn8 plants displayed an approximately 20% reduction in fresh weight compared to wild type after 5 weeks in all light conditions except in red light where they grew to a similar size (Figure 1). psb33 plants, on the other hand, showed similar fresh weight as wild type in fluorescent white and red light while a dramatic and statistically significant (one-way ANOVA, p<0.001) decrease in blue light was observed. The fresh weight of the psb33 plants grown under a combination of red and blue light displayed an intermediate reduction in size compared to those cultivated in white or blue light. The growth phenotype of psb33 in the blue light did not correlate with changes in chlorophyll or carotenoid content (Table 1).

These data point to the fact that the slow-growth phenotype of the psb33 mutant is related to sensitivity towards blue light. Further, the phenotype appeared not to be a consequence of impaired state transition, since plants lacking the kinases STN7 and STN8 (stn7stn8 plants) did not respond similarly as psb33 to the tested light regimes.

Chlorophyll fluorescence characteristics of psb33 in blue and red light

Next, we determined the photosynthetic performance of plants grown under white, blue, and red or a combination of blue and red light (Figure 2A). The maximum quantum efficiency of Photosystem II (Fv/Fm) was similar between the control plants (wild type and the stn7stn8) and showed only small variations in the different light conditions. Following our previous results (Fristedt et al., 2015), Fv/Fm in psb33 was slightly, yet statistically (one-way ANOVA, p<0.001) lower than in wild type in white light. However, in blue, red, and combined blue/red light, the Fv/Fm values were reduced by approximately 20% in psb33, indicating decreased PSII efficiency in these conditions (Figure 2A). In repeated experiments, Fv/Fm was consistently lower in psb33 plants grown in blue light compared to plants grown in red light. However, this difference was not statistically significant in all experiments (data not shown). The performance index parameter (PiABS) is an overall parameter of plant vitality and a sensitive index to evaluate stress (Kalaji et al., 2014). PiABS takes into account the concentration of active reaction centers, the force of the light reactions, as well as the force of the dark reactions (Strasser et al., 2000). Although there were some variability
between replicates, *stn7stn8* plants largely displayed similar PI$_{ABS}$ as the wild type in all light conditions (Figure S3). However, *psb33* plants grown in blue, red, and the combination of blue and red lights displayed a decrease in PI$_{ABS}$ in comparison to *psb33* plants grown in white fluorescence light.

To further explore the response of *psb33* to blue and red light. Plants grown in fluorescent white light were transferred to blue and red light and $Fv/Fm$ was determined at intervals for up to three days (Figure 2B and C). Recovery of the plants was assessed by monitoring the chlorophyll fluorescence after the transfer of plants back to white light. Both blue and red light-induced a fast decrease of the $Fv/Fm$ ratio and reached similar values as in the long term acclimated plants after three days. Following three days of recovery in white light, $Fv/Fm$ completely reverted to steady-state values again, showing that these effects were reversible (Figure 2B and C).

The Chl fluorescence phenotype of *psb33* is not found in other related photosynthetic mutants

To gain insight into the mechanism behind the strong response of the *psb33* plants to blue and red light, we compared the performance of *psb33* under these light treatments to that of well-characterized photosynthesis mutants. Specifically, mutants lacking components of the photosynthetic machinery associated with excitation energy transfer were selected and compared to *psb33* (Figure 2D and E). The kinases STN7 and STN8, and phosphatase TAP38 regulate the phosphorylation status of PSII core and LHCII proteins (Bellafiore et al., 2005; Bonardi et al., 2005; Pribil et al., 2010; Shapiguzov et al., 2010). The *stn7*, the *stn8*, the *stn7stn8*, and the *tap38* mutants have reduced state transition capacity and differ in sensitivity to high or fluctuating light (Fristedt et al., 2009; Tikkanen et al., 2010). The *lhcb1* and the *lhcb2* mutants, corresponding to plants deficient in the Lhcb1 and the Lhcb2, respectively, show altered antenna and thylakoid structure, impaired state transition, and reduced NPQ (Pietrzykowska et al., 2014). The *npq4* mutant is devoid of the protein PsbS and lacks the qE component of NPQ, is sensitive to photoinhibition (Li et al., 2002) and fluctuating light (Külheim et al., 2002). PsbS has also been proposed to play a role in energy distribution between the two photosystems (Jajoo et al., 2014). PsbW plays an important
role in PSII function, and the corresponding \textit{psbw} mutant shows destabilized PSII supercomplex organization, decreased phosphorylation of PSII core proteins, and faster changes of the redox state of the plastoquinone pool (García-Cerdán et al., 2011). \textit{psal} mutants lack a PSI component, show reduced PSI electron transport rate, normal phosphorylation of LHCII but inability to increase the PSI antenna size in State II light (Lunde et al., 2000). Finally, PGR5 encodes a protein previously thought to be involved in cyclic electron transport around PSI (Munekage et al., 2002), but more recently shown to be involved in regulating proton flux across the thylakoid membrane (Kanazawa et al., 2017; Suorsa et al., 2012). The \textit{pgr5} mutant is unable to induce NPQ, is sensitive to high light, which results in PSI damage (Munekage et al., 2002), shows stunted growth in normal conditions, and is unable to cope with fluctuating light resulting in wilting (Tikkanen et al., 2010).

Comparing the \textit{Fv/Fm} parameter of all these mutant lines and wild types in response to blue and red light, it is clear that the \textit{psb33} mutant was the only line showing a substantial reduction in photosynthetic performance (Figure 2D and E). There was no statistical difference in \textit{Fv/Fm} among the \textit{psb33} plants kept for three or seven days in blue or red light. These results indicated that the observed phenotype of \textit{psb33} is not connected to thylakoid ΔpH-dependent regulation mechanisms since \textit{pgr5} and \textit{npq4} were unaffected under the tested light regimes. The results further strengthened the notion that the phenotype of \textit{psb33} cannot be explained by the lack of photosystem energy distribution-dependent mechanisms since none of the included mutants with impaired ability to phosphorylate PSII core or with altered antenna proteins displayed stress symptoms under red or blue light.

\textit{psb33} plants display altered phytohormone levels in blue and red light

Plant growth restrictions are commonly associated with defective hormone control. Therefore, we tested if the response of \textit{psb33} plants to blue and red light was accompanied by changes in leaf phytohormone levels (Figure 3). Our analysis specifically targeted hormones involved in growth and stress signaling: jasmonates (including JA, isoleucine-conjugated JA (JA-Ile), and 12-oxo-phytodienoic acid (\textit{cis}-OPDA)), salicylic acid (SA), indole acetic acid (IAA), and IAA derivatives. SA levels were 3.6 and 5.5 times lower (p<0.01, one-
way ANOVA, Bonferroni-Holm posthoc test) in the mutant compared to wild type in blue and red light, respectively. However, levels were relatively low in both the wild type and psb33 in all conditions (Figure 3F). The only statistically significant hormonal change exclusively apparent in psb33 grown in blue light was a strong accumulation of aspartic acid conjugated IAA (IAA-Asp) (Figure 3C).

*Blue light induces changes to psb33 thylakoid ultrastructure*

To further explore the psb33 growth phenotype and to resolve whether it relates to morphological changes at the microscopic level, the thylakoid ultrastructure of psb33 plants grown in white, blue, and red light was examined by transmission electron microscopy (Figure 4). No difference in thylakoid structure was observed between wild type and psb33 plants grown in fluorescent white- or red light. Thylakoids of psb33 grown in blue light were found to be swollen, showing large luminal spaces at non-appressed regions of the thylakoid membrane (Figure 4B). Such swollen thylakoids could be observed in a few chloroplasts also from wild type plants taken from blue light. However, virtually all thylakoids from psb33 plants from blue light displayed such abnormal structures.

*UV-A light causes the psb33 growth phenotype*

Next, we set out to more specifically delineate the light wavelengths causing the deleterious growth phenotype of psb33 in blue light. Three blue light conditions provided by three different LEDs were tested at the PFD of 120 µmol m$^{-2}$ s$^{-1}$: [400], [420,440], and [400,420,440] nm (Figure 5). psb33 plants cultivated under only 400 nm light only reached 12% of wild type fresh weight at the end of the growth period (Figure 5A and B), while psb33 plants in [420,440] nm and [400,420,450] nm reached 66% and 40%, respectively, of the weight of wild type.

Due to the fact that the 400 nm LED lamp emits light ranging from 391 nm to 421 nm, with a peak at 404-405 nm, we suspect that light in the UVA region is the main cause of the dwarf phenotype of psb33 grown in blue light (Figure 1, Figure 5, Figure S4). Growing plants in 385 nm UVA light corroborated this finding: psb33 plants displayed strong growth restriction
compared to the wild type in this light condition (Figure S5). Chlorophyll \textit{a} fluorescence measurements showed a drastic reduction in quantum efficiency (\(F_{v}/F_{m}\)) of PSII and PI_{ABS} in \textit{psb33} plants grown in lights with UVA (Figure 5C and S5). The \(F_{v}/F_{m}\) ratio of \textit{psb33} plants grown in [420,450] nm was significantly higher (one-way ANOVA, \(p<0.01\)) than in plants grown in [400] nm or [400,420,440] nm (Figure 5C). Thus, plants cultivated in light conditions lacking UVA wavelengths performed better in terms of photosynthetic performance than plants grown in lights containing UVA.

\textit{PSII core protein levels are reduced in \textit{psb33}}

The low \(F_{v}/F_{m}\) measured in the \textit{psb33} mutant indicated decreased PSII efficiency and possibly damage to the PSII complex. We, therefore, examined potential photodamage to PSII in \textit{psb33} grown in various blue light conditions. To investigate if PSII and PSI protein levels were influenced by the quality of light, whole-leaf proteins were extracted from plants grown in either fluorescent white light, blue light with or without the 400 nm UVA component (Figure S4) ([400,420,440] nm or [420,440] nm respectively), and red light. Further, the fresh weight of plants was determined, and whole leaf proteins extracted from wild type and \textit{psb33} plants grown under blue and red light in combination with far-red light ([400,20,440,730] nm or [630,660,730] nm, respectively) (Figure S7). Far-red light was included in the experimental setup since it predominantly excites PSI. The far-red light treatment could thus reveal if increased excitation pressure of PSI would balance the relative energy distribution between the two photosystems and reduce photodamage. Extracted whole leaf proteins were separated on SDS-PAGE, immunoblotted and labeled with antibodies specific for PSII (D1 and D2), PSI (PsaA and PsaB), LHClI (Lhcb1 and Lhcb2) and its phosphorylated forms (Lhcb1-P and Lhcb2-P) and Rubisco large subunit (RbcL) (Figure 6A). PsaA and PsaB levels varied in the different light conditions but were largely similar between wild type and \textit{psb33}. So was the abundance of RbcL. In agreement with the impaired state transition phenotype of \textit{psb33}, phosphorylation of Lhcb1 and Lhcb2 was almost completely lost in the \textit{psb33} mutant in blue light. Notably, in red light and light supplemented with far-red, Lhcbs were phosphorylated to a certain level in \textit{psb33} plants.
Furthermore, very little phosphorylation of Lhcb proteins was observed in psb33 in fluorescent white light, corroborating our previous findings (Fristedt et al., 2017). Our compiled data thus indicated that the redistribution of energy between the two photosystems through re-arrangement of the LHCII light-harvesting antenna is impaired in psb33. Yet, this deficiency cannot alone explain the blue light growth phenotype. The PSII core proteins D1 and D2 were both reduced in psb33 in blue light conditions. The corresponding reduction in protein abundance occurred in psb33 exposed to 400 nm LED light, whereas plants exposed to [420,440] nm light displayed higher levels of D1 than plants from only [400] nm or [400/420/440] nm (Figure 6A and B).

**PSII electron transport properties are modified in psb33**

The reduced levels of the D1 and D2 proteins in psb33 cultivated under UVA light may indicate an altered redox state of the acceptor side of PSII. To investigate this, we performed flash-induced variable fluorescence decay and thermoluminescence measurements in isolated PSII membranes from wild type and psb33 plants. Analysis of the flash-induced fluorescence decay kinetics demonstrated faster electron transfer from $Q_A$ to $Q_B$ in psb33 mutant (Figure 7A, left panel) (Mamedov et al., 2000; Roose et al., 2010; Vass et al., 1999). Also, thermoluminescence measurements, which are a useful complement to the flash-induced fluorescence decay measurements (Ducruet and Vass, 2009; Vass and Govindjee, 1996), also showed the presence of only a slightly up-shifted B-band (which represents the $Q_B^\cdot \rightarrow S_2$ state recombination) at 38 °C. This is different compared to a mixture of the Q-band (which represents the $Q_A^\cdot \rightarrow S_2$ state recombination) and B-band, present in thylakoids from wild type at 14 °C and 36 °C (Figure 7B, left panel). Thus, both measurements confirm the presence of more oxidized acceptor side of PSII and higher redox potential of $Q_B$ in psb33 mutant.

The fluorescence decay kinetics and thermoluminescence bands measured in the presence of DCMU (and in case of thermoluminescence indicating only the $Q_A^\cdot \rightarrow S_2$ state recombination), were virtually the same in the wild type and psb33 mutant (Figure 7A and B, respectively, right panel). This indicated that the redox properties of $Q_A$ and the water oxidizing complex on the donor side of PSII were not affected in the psb33 mutant. This was
also corroborated by EPR measurements of the $S_2$ state multiline signal which were found similar in wild type and psb33 mutant (Figure 7D).

Interestingly, Cytochrome b$_{559}$, an integral part and an alternative electron donor/acceptor of the PSII reaction center (Shinopoulos and Brudvig, 2012) was found to be predominantly in the low potential form in the psb33 mutant (Figure 7E) again, pointing out the more oxidized acceptor side in PSII. However, the overall amount of the PSII reaction centers, as judged from the Tyrosine D radical on the Chl basis, was only slightly diminished in the psb33 mutant (Figure 7C).

**psb33 ROS levels are unaffected by UVA light**

The redox potential of Q$_B$ is important for the regulation of electron transfer both for forward and backward electron transfer, which functions as a protective mechanism for PSII (Kato et al., 2016). The modulated potential of Q$_B$ in combination with elevated excitation pressure on PSII via UVA light, could induce ROS mediated damage to D1 and D2 in psb33 mutant plants. To test this, we investigated if psb33 mutant plants differed in ROS levels compared to wild type plants in UVA light. Plants grown in [400] or [420,450] nm light were stained for hydrogen peroxide using 3,3’-diaminobenzidine and superoxide (O$_2^-$) radicals using nitroblue tetrazolium (NBT). Surprisingly, no visual differences in levels of either H$_2$O$_2$ by DAB or O$_2^-$ by NBT were observed between wild type and psb33 (Figure 8).

**Discussion**

The mechanisms by which plants maintain and optimize photosynthesis in fluctuating light environments are rapidly being unraveled (Cruz et al., 2016; Tikkanen et al., 2010; Tikkanen et al., 2012; Vialet-Chabrand et al., 2017). But as natural light fluctuates in intensity, it also varies considerably in quality, and the influence of light color in photosynthetic regulation has so far largely been overlooked (Allorent and Petroutsos, 2017). In this study, we demonstrate that plants lacking PSB33 are particularly sensitive to light in the UVA spectral region. UVA light was found to induce a dwarf phenotype in psb33 plants, which was associated with diminished PSII protein levels.
**PSB33 has a specific spectral influence on PSII and linear electron flow**

Since the growth phenotype of the *psb33* mutant appeared to be very variable and strictly dependent on light conditions in our different growth facilities, we decided to pay particular emphasis on the light quality in inducing the *psb33* phenotype in this work. By cultivating the mutant in different light settings, it was found that the reduced growth of *psb33* was specifically induced by blue light (Figure 1 and S3), particularly the spectral region that composes the UVA (Figure 5). We, therefore, suggest that the previously reported growth phenotype, reaching approximately 50% the size of the wild type under normal growth condition (Fristedt et al., 2015), was due to the presence of short-wavelength blue light in the light source of the growth chambers.

The slow-growth of *psb33* in blue light was found to be connected to low levels of PSII core proteins D1 and D2 (Figure 6) which inevitably leads to reduced photosynthesis rate. The effect was more pronounced in UVA (400 nm) blue light than in [420,450] nm or [400,420,450] nm blue light at the same total photon flux (Figure 6B). The red light did not cause any difference in D1 or D2 protein abundance between wild type and mutant. It is interesting to note that both blue- and red light-grown plants displayed a similar reduction in maximal PSII quantum yield (*Fv/Fm*) (Figure 2) and PI\(_{ABS}\) (Figure S3). However, *psb33* plants grown in exclusively blue light at 400 nm showed further reduced *Fv/Fm* values (Figure 5C).

Supplementing UVA containing blue light with far-red light rescued the phenotype to some extent both in terms of growth and PSII protein levels (Figure 6A and Figure S7). As far-red light predominately excites PSI, a possible explanation to this rescue is that PSB33 has a role in the linear electron transport chain to balance the redox components between the two photosystems. In the absence of PSB33, an unbalance of redox components in the electron transport chain would initiate the production of ROS, resulting in elevated PSII damage. However, we did not observe an increase of ROS in leaves from plants grown in UVA light that would support an over-excitation of PSII and subsequently increased photodamage. Another, perhaps more likely explanation is that the rescue effect of far-red is a consequence of reduced UVA blue light as the total photon flux was kept constant in the experiments (i.e., 50% of the total light was provided by far-red and 50% by blue light).
When we tested mutants with reported impaired ability for PSII super-structure (psbw), state transitions (lhcb1, lchb2, psal, and stn7/8), cyclic electron transport and proton gradient (pgr5) or NPQ (npq4), none exhibited a slow-growth phenotype comparable to that of psb33 in blue light, indicating that PSB33 has a unique role in blue light perception unrelated to these known pathways to maintain photosynthesis.

Salicylic acid and aspartic acid conjugated indole-3-acetic acid hormone levels differ in psb33 plants exposed to different lights

SA levels are well known to influence plant growth negatively and to be important for light acclimation (Rivas-San Vicente and Plasencia, 2011). Surprisingly, the SA level decreased in response to both blue and red light treatments in psb33, whereas it increased in the wild-type (Fig. 3F). SA and glutathione interplay to regulate antioxidant response through ROS scavenging (Herrera-Vasquez et al., 2015). The low levels of SA in psb33 could accordingly explain the accumulation of different ROS in the mutant line, as previously reported (Fristedt et al., 2015). However, we could not observe any differences between wild type and psb33 in, or the absence of, UVA light ([400] nm and [420,450] nm, respectively) in terms of H$_2$O$_2$ and superoxide radicals (Fig. 8), suggesting that changed SA and ROS levels did not contribute to the deleterious effect of UVA light. The only hormonal change exclusively observed in psb33 grown in blue light was a strong accumulation of aspartic acid conjugated IAA (IAA-Asp) (Figure 3C). This is believed to be an inactive form of IAA that is destined for catabolism (Ludwig-Muller, 2011; Woodward and Bartel, 2005).

Psb33 plants grown in blue light show an abnormal thylakoid ultrastructure.

PSB33 is primarily located in non-appressed thylakoid regions (Fristedt et al., 2015; Fristedt et al., 2017; Kato et al., 2017). Quality control of PSII is dependent on reversible thylakoid structure dynamics (Yamamoto, 2016). Light induces a reversible swelling and unstacking of the thylakoids, which are necessary for proper PSII repair and protects PSII from damage (Khatoon et al., 2009). Furthermore, the increased surface area of the membranes influences its fluidity, important for lateral movement of proteins and other compounds
within the membrane regulating NPQ, state transition, and PSII repair (Yamamoto, 2016). Integrated regulation of the many processes involved in the dynamics of the thylakoid membrane is not yet fully understood (Tikkanen and Aro, 2014). It has been shown that the PSII-LHCII supercomplex phosphorylation level affects the distance between the grana stacks as well as the size and suggested that complexes in opposite orientation repel each other, causing unstacking of the grana (Fristedt et al., 2009). Thus, the reduced levels of PSII (D1 and D2) observed in psb33 mutant plants exposed to blue light (Figure 6), could explain changes to the thylakoid structure of psb33, seen by transmission electron microscopy (Figure 4). The distinctly swollen stroma lamella might negatively influence the rate of movement of proteins and compounds, restrict PSII quality control, and enhance damage to the complex (Kirchhoff, 2014). The altered PSII superstructure and PSII level in psb33 might have further enhanced the growth phenotype.

**UVA light reduces Photosystem II proteins in psb33.**

Two previous observations have been linked to blue- and UVA light that can explain psb33 dwarf phenotype. First, the Mn₄CaO₅ can directly absorb UV light and initiate the photoinactivation process (Ohnishi et al., 2005). It could be possible, but not likely, that PSB33 protects the Mn cluster, due to restricted access by the OEC proteins. However, our data on intra PSII electron flow do not support this hypothesis (Figure 7). Second, blue- and UVA light-mediated signals have been linked to increased D2 and CP43 gene expression in mature chloroplasts (Christopher and Mullet, 1994) and this is consistent with our results: we observed an up-regulation of D1 and D2 PSII proteins in wild type plants exposed to blue/UVA light (Figure 6A). In contrast, D1 and D2 were down-regulated in psb33 mutant plants in blue/UVA.

PSB33 location is largely in non-appressed thylakoid regions, and with its head domain exposed to the stroma (Fristedt et al., 2015), and is part of a larger protein complex (Fristedt et al., 2017). PSB33 is likely part of a signaling mechanism involved in the rejuvenation of PSII. A hypothetical scenario would be that this mechanism is either triggered by UVA light or develops as a consequence of plant exposure to blue and UV light, possibly as a result of re-adjusted redox regulation. Such a signaling cascade may eventually induce a
conformational change on PSB33, which triggers downstream signaling for transcription of various PSII proteins.

Conclusions

The accumulated data show that PSB33 has an important role in sustaining a functional PSII pool in blue light, especially in UVA light. Although the severe growth phenotype of psb33 plants in blue light is connected to misregulated phosphorylation and migration of Lhcb proteins, this appears not to be a causal relationship. The exact molecular mechanism of how PSB33 protein senses UV-light and initiates the photosynthetic response remains to be investigated.
Data Availability Statement
The data supporting the findings of this study are available from the corresponding author, Björn Lundin Burmeister, upon request.

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Author contribution
AN and BL designed the experiments. AN, DB, and BL performed light experiments and growth of plants. AN and BL performed pigment, chlorophyll a fluorescence, thylakoid membrane preparation, SDS-PAGE, immunoblotting, and ROS determination. AP and ON performed hormonal analysis. OJ performed transmission electron microscopy. FM performed thermoluminescence and low-temperature electron paramagnetic resonance spectroscopy. All authors contributed to the analysis of results and writing of the manuscript.

Conflict of Interest
Björn Burmeister is employed at AstraZeneca, Respiratory & Immunology at the time of publication and declares that there is no conflict of interest that has effected this publication.
References

Adamska I, Klöppstech K, Ohad I. 1992. UV light stress induces the synthesis of the early light-inducible protein and prevents its degradation. Journal of Biological Chemistry 267, 24732-24737.

Allen JF, Bennett J, Steinback KE, Arntzen CJ. 1981. Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature 291, 25-29.

Allorent G, Lefebvre-Legendre L, Chappuis R, Kuntz M, Truong TB, Niyogi KK, Ulm R, Goldschmidt-Clermont M. 2016. UV-B photoreceptor-mediated protection of the photosynthetic machinery in Chlamydomonas reinhardtii. Proceedings of the National Academy of Sciences of the United States of America 113, 14864-14869.

Allorent G, Petroutsos D. 2017. Photoreceptor-dependent regulation of photoprotection. Current Opinion in Plant Biology 37, 102-108.

Aro E-M, Virgin I, Andersson B. 1993. Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1143, 113-134.

Bellaire F, Barneche F, Pelletier G, Rochaix JD. 2005. State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. Nature 433, 892-895.

Bonardi V, Pesaresi P, Becker T, Schleiff E, Wagner R, Pfannschmidt T, Jahns P, Leister D. 2005. Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. Nature 437, 1179-1182.

Cazzaniga S, Dall’Osto L, Kong SG, Wada M, Bassi R. 2013. Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photooxidative stress in Arabidopsis. The Plant Journal 76, 568-579.

Chaiwanon J, Wang W, Zhu JY, Oh E, Wang ZY. 2016. Information Integration and Communication in Plant Growth Regulation. Cell 164, 1257-1268.

Chen G, Allahverdiyeva Y, Aro EM, Styring S, Mamedov F. 2011. Electron paramagnetic resonance study of the electron transfer reactions in photosystem II membrane preparations from Arabidopsis thaliana. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1807, 205-215.

Christie JM, Blackwood L, Petersen J, Sullivan S. 2015. Plant Flavoprotein Photoreceptors. Plant and Cell Physiology 56, 401-413.

Christopher DA, Mullet JE. 1994. Separate Photosensory Pathways Co-Regulate Blue Light/Ultraviolet-A-Activated psbD-psbC Transcription and Light-Induced D2 and CP43 Degradation in Barley (Hordeum vulgare) Chloroplasts. Plant Physiol 104, 1119-1129.

Cruz JA, Savage LJ, Zegarac R, Hall CC, Satoh-Cruz M, Davis GA, Kovac WK, Chen J, Kramer DM. 2016. Dynamic Environmental Photosynthetic Imaging Reveals Emergent Phenotypes. Cell Systems 2, 365-377.

Daudi A, O’Brien JA. 2012. Detection of Hydrogen Peroxide by DAB Staining in Arabidopsis Leaves. Bio-protocol 2, e263.

Ducruet JM, Vass I. 2009. Thermoluminescence: experimental. Photosynthesis Research 101, 195-204.

Flokova K, Tarkowska D, Miersch O, Strnad M, Wasternack C, Novak O. 2014. UHPLC-MS/MS based target profiling of stress-induced phytohormones. Phytochemistry 105, 147-157.
Folta KM, Kaufman LS. 1999. Regions of the Pea Lhcb1*4 Promoter Necessary for Blue-Light Regulation in Transgenic Arabidopsis. Plant Physiology 120, 747-756.

Fristedt R, Herdean A, Bley-Haas CE, Mamedov F, Merchant SS, Last RL, Lundin B. 2015. PHOTOSYSTEM II PROTEIN33, a protein conserved in the plastid lineage, is associated with the chloroplast thylakoid membrane and provides stability to photosystem II supercomplexes in Arabidopsis. Plant Physiology 167, 481-496.

Fristedt R, Trotta A, Suorsa M, Nilsson AK, Croce R, Aro EM, Lundin B. 2017. PSB33 sustains photosystem II D1 protein under fluctuating light conditions. Journal of Experimental Botany 68, 4281-4293.

Fristedt R, Vener AV. 2011. High Light Induced Disassembly of Photosystem II Supercomplexes in Arabidopsis Requires STN7-Dependent Phosphorylation of CP29. PLoS One 6, e24565.

Fristedt R, Willig A, Granath P, Crevecoeur M, Rochaix JD, Vener AV. 2009. Phosphorylation of photosystem II controls functional macroscopic folding of photosynthetic membranes in Arabidopsis. Plant Cell 21, 3950-3964.

García-Cerdán JG, Kovács L, Tóth T, Kereïche S, Aseeva E, Boekema EJ, Mamedov F, Funk C, Schröder WP. 2011. The PsbW protein stabilizes the supramolecular organization of photosystem II in higher plants. The Plant Journal 65, 368-381.

Goh CH. 2009. Phototropins and chloroplast activity in plant blue light signaling. Plant Signal Behavior 4, 693-695.

Hakala M, Tuominen I, Keränen M, Tyystjärvi T, Tyystjärvi E. 2005. Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of Photosystem II. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1706, 68-80.

Herdean A, Teardo E, Nilsson AK, Pfeil BE, Johansson ON, Unnep R, Nagy G, Zsíros O, Dana S, Solymosi K, Garab G, Szabo I, Spetea C, Lundin B. 2016. A voltage-dependent chloride channel fine-tunes photosynthesis in plants. Nature Communication 7, 11654.

Herrera-Vasquez A, Salinas P, Holuique L. 2015. Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression. Frontiers in Plant Science 6, 171.

Jajoo A, Mekala NR, Tongra T, Tiwari A, Grieco M, Tikkanen M, Aro E. 2016. A voltage-
dependent chloride channel tunes photosynthesis in plants. Nature Communication 7, 11654.

Jones LW, Kok B. 1966. Photoinhibition of chloroplast reactions. I. Kinetics and action spectra. Plant Physiology 41, 1037-1043.

Kalaji HM, Schansker G, Ladle RJ, Goltsiev V, Bosa K, Allahverdiev SI, Brestic M, Bussotti F, Calatayud A, Dabrowski P, Elsheyre NI, Ferroni L, Guidi L, Hogewoning SW, Jajoo A, Misra AN, Nebauer SG, Pancaldi S, Penella C, Poli D, Pollastrini M, Romanowska-Duda ZB, Rutkowska B, Serodio J, Suresh K, Szuce L, Tambussi E, Yannicci M, Zivcak M. 2014. Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. Photosynthesis Research 122, 121-158.

Kanazawa A, Ostedorf E, Kohzuma K, Hoh D, Strand DD, Sato-Cruz M, Savage L, Cruz JA, Fisher N, Frochlich JE, Kramer DM. 2017. Chloroplast ATP Synthase Modulation of the Thylakoid Proton Motive Force: Implications for Photosystem I and Photosystem II Photoprotection. Frontiers in Plant Science 8, 719.

Kato Y, Nagao R, Noguchi T. 2016. Redox potential of the terminal quinone acceptor QB in photosystem II reveals the mechanism of electron transfer regulation. Proceedings of the National Academy of Sciences of the United States of America 113, 620-625.

Kato Y, Sakamoto W. 2014. Phosphorylation of photosystem II core proteins prevents undesirable cleavage of D1 and contributes to the fine-tuned repair of photosystem II. The Plant Journal 79, 312-321.

Kato Y, Yokono M, Akimoto M, Takabayashi A, Tanaka A, Tanaka R. 2017. Deficiency of the Stroma-Lamellar Protein LIL8/PSB33 Affects Energy Transfer Around PSI in Arabidopsis. Plant Cell Physiology 58, 2026-2039.

Khatoun M, Inagawa K, Pospisil P, Yamashita A, Yoshioka M, Lundin B, Horie J, Morita N, Jajoo A, Yamamoto Y, Yamamoto Y. 2009. Quality control of photosystem II: Thylakoid
unstacking is necessary to avoid further damage to the D1 protein and to facilitate D1 degradation under light stress in spinach thylakoids. Journal of Biological Chemistry 284, 25343-25352.

Kirchhoff H. 2014. Diffusion of molecules and macromolecules in thylakoid membranes. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1837, 495-502.

Kirilovsky D, Kerfeld CA. 2013. The Orange Carotenoid Protein: a blue-green light photoreactive protein. Photochemical and Photobiological Sciences 12, 1135-1143.

Kok B. 1956. On the inhibition of photosynthesis by intense light. Biochim Biophys Acta 21, 234-244.

Kühlheim C, Ägren J, Jansson S. 2002. Rapid Regulation of Light Harvesting and Plant Fitness in the Field. Science 297, 91-93.

Li L, Aro EM, Millar AH. 2018. Mechanisms of Photodamage and Protein Turnover in Photoinhibition. Trends in Plant Science 23, 667-676.

Li XP, Muller-Moule P, Gilmore AM, Niyogi KK. 2002. PsbS-dependent enhancement of feedback de-excitation protecst proteosystem II from photoinhibition. Proceedings of the National Academy of Sciences of the United States of America 99, 15222-15227.

Lichtenthaler HK, Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions 11, 591-592.

Long S, Humphries S, Falkowski PG. 1994. Photoinhibition of Photosynthesis in Nature. Annual Review of Plant Physiology and Plant Molecular Biology 45, 633-662.

Ludwig-Muller J. 2011. Auxin conjugates: their role for plant development and in the evolution of land plants. Journal of Experimental Botany 62, 1757-1773.

Lunde C, Jensen PE, Haldrup A, Knoetzel J, Scheller HV. 2000. The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. Nature 408, 613-615.

Mamedov F, Stefansson H, Albertsson P-A, Styring S. 2000. Photosystem II in Different Parts of the Thylakoid Membrane: A Functional Comparison between Different Domains. Biochemistry 39, 10478-10486.

Mekala NR, Suorsa M, Rantala M, Aro EM, Tikkanen M. 2015. Plants Actively Avoid State Transitions upon Changes in Light Intensity: Role of Light-Harvesting Complex II Protein Dephosphorylation in High Light. Plant Physiol 168, 721-734.

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T. 2002. PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis. Cell 110, 361-371.

Ohad I, Kyle DJ, Arntzen CJ. 1984. Membrane protein damage and repair: removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes. The Journal of Cell Biology 99, 481-485.

Ohnishi N, Allakhverdiev SI, Takahashi S, Higashi S, Watanabe M, Nishiyama Y, Murata N. 2005. Two-step mechanism of photodamage to photosystem II: step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. Biochemistry 44, 8494-8499.

Petroutsos D, Tokutsu R, Maruyama S, Flori S, Greiner A, Magneschi L, Cusan J, Kottke T, Mittag M, Hegemann P, Finazzi G, Minagawa J. 2016. A blue-light photoreceptor mediates the feedback regulation of photosynthesis. Nature 537, 563.

Pietrzykowska M, Suorsa M, Semchonok DA, Tikkanen M, Boekema EJ, Aro EM, Jansson S. 2014. The light-harvesting chlorophyll a/b binding proteins Lhcb1 and Lhcb2 play complementary roles during state transitions in Arabidopsis. Plant Cell 26, 3646-3660.

Porra R, Thompson W, Kriedemann P. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta (BBA) - Bioenergetics 975, 384-394.

Pribil M, Pesaresi P, Hertle A, Barbato R, Leister D. 2010. Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow. PLoS Biology 8, e1000288.

Rintamäki E, Martinsoo P, Pursiheimo S, Aro EM. 2000. Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts. Proceedings of the National Academy of Sciences of the United States of America 97, 11644-11649.
Rivas-San Vicente M, Plasencia J. 2011. Salicylic acid beyond defence: its role in plant growth and development. Journal of Experimental Botany 62, 3321-3338.

Roose JL, Yocum CF, Popelkova H. 2010. Function of PsbO, the photosystem II manganese-stabilizing protein: probing the role of aspartic acid 157. Biochemistry 49, 6042-6051.

Saadatian-Elahi M, Slimani N, Chajes V, Jenab M, Goudable J, Biessy C, Ferrari P, Byrnes G, Autier P, Peeters PH, Ocke M, Bueno de Mesquita B, Johannson I, Hallmans G, Manjer J, Wirfalt E, Gonzalez CA, Navarro C, Martinez C, Amiano P, Suarez LR, Ardanaz E, Tjonneland A, Halkjaer J, Overvad K, Jakobsen MU, Berrino F, Pala V, Palli D, Tumino R, Vineis P, Santucci de Magistris M, Spencer EA, Crowe FL, Bingham S, Khaw KT, Linsell J, Rohrmann S, Boeing H, Noethlings U, Olsen KS, Skeie G, Lund E, Trichopoulou A, Oustoglou E, Clavel-Chapelon F, Riboli E. 2009. Plasma phospholipid fatty acid profiles and their association with food intakes: results from a cross-sectional study within the European Prospective Investigation into Cancer and Nutrition. The American Journal of Clinical Nutrition 89, 331-346.

Sarvikas P, Hakala M, Patsiška E, Tyystjarvi T, Tyystjarvi E. 2006. Action spectrum of photoinhibition in leaves of wild type and npq1-2 and npq4-1 mutants of Arabidopsis thaliana. Plant Cell Physiology 47, 391-400.

Schellenberger Costa B, Jungandreas A, Jakob T, Weisheit W, Mittag M, Wilhelm C. 2013. Blue light is essential for high light acclimation and photoprotection in the diatom Phaeodactylum tricornutum. Journal of Experimental Botany 64, 483-493.

Scheller H, Haldrup A. 2005. Photoinhibition of photosystem I. Planta 221, 5-8.

Shapiguzov A, Ingelsson B, Samol I, Andres C, Kessler F, Rochaix JD, Vener AV, Goldschmidt-Clermont M. 2010. The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America 107, 4782-4787.

Shinopoulos KE, Brudvig GW. 2012. Cytochrome b559 and cyclic electron transfer within photosystem II. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1817, 66-75.

Strasser RJ, Srivastava A, Tsimilli M. 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. Probing photosynthesis: mechanism, regulation & adaptation.

Strasser RJ, Tsimilli-Michael M, Srivastava A. 2004. Analysis of the Chlorophyll a Fluorescence Transient. In: Papageorgiou GC, Govindjee, eds. Chlorophyll a Fluorescence: A Signature of Photosynthesis. Dordrecht: Springer Netherlands, 321-362.

Suorsa M, Jarvi S, Greico M, Nurmi M, Pietrzykowska M, Rantal a M, Kangasjarvi S, Paakkarinen V, Tikkanen M, Jansson S, Aro EM. 2012. PROTON GRADIENT REGULATION 5 is essential for proper acclimation of Arabidopsis photosystem I to naturally and artificially fluctuating light conditions. Plant Cell 24, 2934-2948.

Takahashi S, Milward SE, Yamori W, Evans JR, Hillier W, Badger MR. 2010. The solar action spectrum of photosystem II damage. Plant Physiology 153, 988-993.

Tikkanen M, Aro EM. 2014. Integrative regulatory network of plant thylakoid energy transduction. Trends in Plant Science 19, 10-17.

Tikkanen M, Greico M, Kangasjarvi S, Aro EM. 2010. Thylakoid protein phosphorylation in higher plant chloroplasts optimizes electron transfer under fluctuating light. Plant Physiol 152, 723-735.

Tikkanen M, Greico M, Nurmi M, Rantal a M, Suorsa M, Aro E-M. 2012. Regulation of the photosynthetic apparatus under fluctuating growth light. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 3486-3493.

Tikkanen M, Nurmi M, Kangasjärvi S, Aro E-M. 2008. Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1777, 1432-1437.

Trotta A, Suorsa M, Rantal a M, Lundin B, Aro EM. 2016. Serine and threonine residues of plant STN7 kinase are differentially phosphorylated upon changing light conditions and specifically influence the activity and stability of the kinase. The Plant Journal 87, 484-494.

Wang H, Wang H. 2014. Phytochrome Signaling: Time to Tighten up the Loose Ends. Molecular Plant 8, 540-551.

Vass I, Govindjee. 1996. Thermoluminescence from the photosynthetic apparatus. Photosynthesis Research 48, 117-126.
Vass I, Kirilovsky D, Etienne AL. 1999. UV-B radiation-induced donor- and acceptor-side modifications of photosystem II in the cyanobacterium Synechocystis sp. PCC 6803. Biochemistry 38, 12786-12794.
Vialet-Chabrand S, Matthews JSA, Simkin AJ, Raines CA, Lawson T. 2017. Importance of Fluctuations in Light on Plant Photosynthetic Acclimation. Plant Physiol 173, 2163-2179.
Volgusheva A, Kruse O, Styring S, Mamedov F. 2016. Changes in the Photosystem II complex associated with hydrogen formation in sulfur deprived Chlamydomonas reinhardtii. Algal Research 18, 296-304.
Woodward AW, Bartel B. 2005. Auxin: Regulation, Action, and Interaction. Annals of Botany 95, 707-735.
Yamamoto Y. 2016. Quality Control of Photosystem II: The Mechanisms for Avoidance and Tolerance of Light and Heat Stresses are Closely Linked to Membrane Fluidity of the Thylakoids. Frontiers in Plant Science 7, 1136.
Yoshioka-Nishimura M, Yamamoto Y. 2014. Quality control of Photosystem II: The molecular basis for the action of FtsH protease and the dynamics of the thylakoid membranes. Journal of Photochemistry and Photobiology B: Biology 137, 100-106.
Zavafer A, Chow WS, Cheah MH. 2015. The action spectrum of Photosystem II photoinactivation in visible light. Journal of Photochemistry and Photobiology B: Biology 152, 247-260.
Tables

Table 1. Pigment composition in plants grown in different lights.

| Pigment Composition | Total chlorophyll (mg g\(^{-1}\)) | Carotenoids (mg g\(^{-1}\)) | Chl a/b |
|---------------------|-----------------------------------|-----------------------------|---------|
|                     | wt      | psb33  | wt      | psb33  | wt      | psb33  |
| Fluorescent white   | 1.11±0.03 | 0.92±0.07** | 0.23±0.01 | 0.18±0.02** | 3.97±0.07 | 3.85±0.03** |
| Blue                | 0.87±0.07 | 0.74±0.08*  | 0.18±0.01 | 0.16±0.02  | 4.71±0.07 | 4.33±0.42  |
| Red                 | 0.96±0.23 | 0.96±0.09 | 0.20±0.05 | 0.21±0.02 | 4.61±0.09 | 4.63±0.20 |
| Blue and red        | 1.07±0.09 | 1.07±0.09 | 0.22±0.02 | 0.23±0.02 | 4.57±0.05 | 4.46±0.13 |

Table 1. Pigment composition was determined in leaf tissue from five-week-old plants. Plants were grown for two weeks in white fluorescent light and then transferred to LED chambers emitting light of different wavelengths of either blue [400,420,440] nm or red [630,650] nm light, or a combination of the two and grown for additional three weeks. Control plants were kept in white fluorescent light throughout the growth period. Asterisks indicate statistically significantly different to wild type under the specified growth condition (*p<0.05, **p<0.01 Student’s t-test, n=6).
Figure legends

Figure 1. **The growth of the psb33 is strongly inhibited by blue monochromatic light.** Plants were grown for five weeks in white fluorescent light at 120 µmols m$^{-2}$s$^{-1}$, or alternatively, grown for two weeks in white fluorescent light and then transferred to blue or red monochromatic light, or a combination of the two (left panel), and grown for additional three weeks before photos were taken (mid panel) and fresh weight (FW) determined (right panel). Percentage values in the right panel indicate relative size to wild type (wt). Means and standard deviation are shown (n=11-24). Letters indicate a statistically significant difference between groups (one-way ANOVA, Bonferroni-Holm posthoc test, p<0.05).

Figure 2. **Fv/Fm in plants under blue and red monochromatic light.** A. Plants were grown for five weeks in fluorescent white light at 120 µmols m$^{-2}$s$^{-1}$, or alternatively, grown for two weeks in fluorescent white light and then transferred to LED growth chambers emitting light in different wavelengths of either blue [400,420,440] nm or red [630,650] nm light, or a combination of the two, and grown for additional three weeks before Fv/Fm was determined (n=14-20). B and C. Plants were grown for four weeks in fluorescent white light and then transferred LED growth chambers emitting light in different wavelengths of either blue [400,420,440] nm (B) or red [630,660] nm light (C) and kept for three days, then returned to fluorescent white light for three days. Fv/Fm values were determined at regular intervals (n=12). D and E Plants were grown for four weeks in fluorescent white light and then transferred LED growth chambers emitting light in different wavelengths of either blue [400/420/440] nm (D) or red [630/660] nm light (E) and Fv/Fm was measured before (0), 3 and 7 days after transfer (n=12-15). N.D. not determined. Means and standard deviation are shown.

Figure 3. Changes in phytohormone levels are observed in psb33 grown in blue or red monochromatic light. Hormones levels were determined in leaf tissue (fresh weight, FW) from plants grown in fluorescent white light (FL) or under LED emitting light in different wavelengths of blue [400,420,440] nm or red [630,660] nm light. Means and standard deviation are shown (n=4). Asterisks indicate a statistically significant difference in the
mutant line \((psb33)\) versus the wild type \((wt)\) as determined by one-way ANOVA with Bonferroni-Holm posthoc test \((p<0.01)\). A indole acetic acid \((IAA)\); B oxindole-3-IAA \((oxIAA)\); C asparagine conjugated IAA \((IAA-Asp)\); D glutamate conjugated IAA \((IAA-Glu)\); E abscisic acid \((ABA)\); F salicylic acid \((SA)\); G jasmonic acid \((JA)\); H isoleucine conjugate JA \((JA-Ile)\); I and 12-oxo-phytodienoic acid \((cis-OPDA)\).

**Figure 4. Thylakoid ultrastructure of psb33 and wild type plants grown in different lights.** Representative transmission electron micrographs are shown for leaf chloroplasts from five-week-old wild type \((wt)\), and psb33 mutant plants fixed two hours after the onset of illumination. The thylakoids of the wild type and psb33 mutant are similar when grown in fluorescent white light or under LED emitting red light in the wavelength of \([630,660] \text{ nm}\). When grown under LED emitting blue light \([400,420,450] \text{ nm}\), psb33 displays swollen thylakoid, rarely observed in the wild type. Scale bars, 2 \(\mu\text{m}\) \((A)\) and 500 \(\text{nm}\) \((B)\).

**Figure 5. psb33 plants are sensitive to UVA light.** Plants were grown for two weeks in fluorescent white light and then transferred to LED emitting blue light in different wavelengths \([400]\) or \([400,420]\) or \([400,420,440] \text{ nm}\) but with similar intensity \((120 \text{ PFD})\) where they were grown for three more weeks before photos were taken \((A)\), fresh weight determined \((B)\), and \(Fv/Fm\) measured \((C)\). Means and standard deviation are shown where \(n=13-15\) and \(n=18-21\), in \(B\) and \(C\) respectively. Asterisks indicate statistically significantly different to wild type under the specified growth condition or between samples marked by brackets \(\text{(one-way ANOVA, Bonferroni-Holm posthoc test, } p<0.01)\). Data used for statistical analysis of fresh weight were transformed \((\text{square root})\) to meet equal variance.

**Figure 6. Western blots of total protein extracts from plants grown in different light conditions.** Whole leaf protein extracts were prepared from plants grown for three weeks in either fluorescent white light or under LED emitting light in different wavelengths of \((A)\) blue light \([400,420,440]\) or \([420,440] \text{ nm}\), red light \([630,650] \text{ nm}\) and blue and red light supplemented with far-red light \([400,420,440,730] \text{ nm}\) or \([630,650,730] \text{ nm}\) and \((B)\) blue light \([400], [420,440] \text{ or } [400,420,440] \text{ nm}\). Proteins were separated with SDS-PAGE, and loading was based on chlorophyll content.
Figure 7. Analysis of electron transport components in photosystem II from thylakoid membranes from wt (black traces/spectra) and psb33 mutant (red traces/spectra). A Flash-induced fluorescence decay kinetic in the absence (left panel) or the presence of 20 μM DCMU (right panel). B Thermoluminescence glow curves in the absence (left panel) or the presence of 40 μM DCMU (right panel). EPR measurements of the C, Tyrosine D radical, D, the S₂ state of the water oxidizing complex and E, the oxidized form of Cytochrome b₅₅₉ in the g₂ region before (dotted line) and after illumination at 77 K (solid line). EPR conditions: microwave frequency: 9.35 GHz, microwave power 1.3 μW and temperature 15 K for C, 10 mW and 7 K for D, and 5 mW and 15 K for E.

Figure 8. ROS staining of plants grown in different wavelengths of blue light. 7-days-old seedlings were transferred from fluorescent white light to LED plant growth chambers emitting light in different wavelengths of blue light [400] or [420,450] nm and grown for 2 weeks before staining against H₂O₂ (3,3'-Diaminobenzidine, DAB) and superoxide radicals (nitroblue tetrazolium, NTB). The photo show representative plants from 5-8 stained plants.
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