

Response of photosynthetic apparatus in *Arabidopsis thaliana* L. mutant deficient in phytochrome A and B to UV-B

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Abstract

The effects of UV-B radiation (1 W m⁻², 1 and 2 h) on PSII activity, chloroplast structure, and H₂O₂ contents in leaves of 26-d-old *Arabidopsis thaliana phyA phyB* double mutant (DMut) compared with the wild type (WT) were investigated. UV-B decreased PSII activity and affected the H₂O₂ content in WT and DMut plants grown under white light (WL). The chloroplast structure changes in DMut plants exposed to UV were more significant than that in WT. Reductions in maximal and real quantum photochemical yields and increase in the value of thermal dissipation of absorbed light energy per PSII RC and the amount of Q_B-nonreducing centers of PSII were bigger in mutant compared to WT plants grown both under WL and red light. Such difference in action of UV-B on WT and DMut can be explained by higher content of UV-absorbing pigments and carotenoids in WT leaves compared with DMut.

Additional key words: chloroplast structure; fluorescence of Chl *a*; photosystem II; phytochrome mutants; stress; ultraviolet.

Introduction

Phytochrome system is a set of plant photoreceptors with phytochrome signaling system, which includes different types of phytochromes (Phy) (5 in *Arabidopsis* and 3 types in rice plants). It is known that PhyB is a key red light (RL) sensor in green plants, which scans RL/far-red light (FRL) ratio of light falling on plants, whereas PhyA predominates in etiolated seedlings and detects the presence of the light in general, first FRL (Casal *et al.* 1998, Quail 2010, Hu *et al.* 2013, Zhao *et al.* 2013). Three other phytochromes: phytochromes C, D, and E are accessory for the RL

perception. The photoreceptor PhyB participates in the reactions induced by short-time RL treatment with relatively low intensity but it also can take part in high irradiance responses induced by long-time RL (Casal *et al.* 1998, 2000, Kreslavski *et al.* 2009). The participation of PhyB was evidenced in adaptation of plants to high and low temperatures, drought, and increased NaCl content (Carvalho *et al.* 2011, Foreman *et al.* 2011, Markovskaya *et al.* 2016) as well as UV radiation (Kreslavski *et al.* 2015, 2016). PhyB is involved in many light-regulated

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Abbreviations: Chl – chlorophyll; DMut – *phytochrome A, phytochrome B* mutant; FRL – far-red light; P_{FR} – active form of phytochrome; PA – photosynthetic apparatus; Phy(P) – phytochrome; RC – reaction center; RL – red light; ROS – reactive oxygen species; WL – white light; WT – wild type.

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processes: the synthesis of photosynthetic pigments and chloroplast development (Zhao *et al.* 2013), the synthesis of some photosynthetic proteins and stomata (Boccalandro *et al.* 2003). However, it usually affects photomorphogenic processes in combination with PhyA (Casal 2000, Sellaro *et al.* 2009, Sineshchekov 2010). For example, both PhyA and PhyB are suggested to play a regulatory role in CO₂ fixation and nonphotochemical quenching of chlorophyll (Chl) fluorescence (Rusaczonok *et al.* 2015).

It is well known that phytochromes take part in the growth and photomorphogenic processes (Quail 2010, Sineshchekov 2010). Less is known about the role of Phy in plant resistance to environmental stress and tolerance (Carvalho *et al.* 2011). Meanwhile, Phy system is likely one of the pathways to regulate the photosynthetic apparatus (PA) tolerance to environmental stressful factors. There are some our and literature data on relationship of the state of Phy system and stress-resistance to high light and UV-radiation, which support this hypothesis (Lingakumar and Kulandaivelu 1993, Thieli *et al.* 1999, Boccalandro *et al.* 2009, Kreslavski *et al.* 2012, 2013a,b, 2015, 2016, Rusaczonok *et al.* 2015). Phy system can take part in regulation of genes sensitive to cold (*COLD-REGULATED*, *COR*); their activation leads to enhanced frost resistance (Franklin and Whitelam 2007).

It is suggested that the Phy system controls stress resistance of the PA by regulation of the activity of light-sensitive genes. It is known, for example, that 10% of genes of *A. thaliana* genome respond to RL or FRL irradiation, which has an influence *via* Phy system (Quail 2007, Jiao *et al.* 2007). It is found that Phy-sensitive genes encode a number of key photosynthetic proteins, enzymes of antioxidant system as well as enzymes of flavonoid and photosynthetic pigments biosynthesis (Franklin and Whitelam 2007, Sellaro *et al.* 2009). Thus, expression levels of *HY5*, *HYH*, *sAPX*, *tAPX*, *APX1*, and *CHS* genes were sensitive to changes in Phy system of *A. thaliana* leaves induced by short-time RL (Kreslavski *et al.* 2016). Note that the *HY5*, *HYH*, and *UVR8* genes are sensitive to

UV-B radiation (Brown and Jenkins 2008). The fact that Phy system affects the contents of the photosynthetic pigments is consistent with a number of experiments, which demonstrated that normal *A. thaliana* seedlings greening does not occur if mutants deficient in all five types of Phy are used (Strasser *et al.* 2010, Hu *et al.* 2013).

Phy mutants of *A. thaliana* are widely used in the plant experiments, especially the role of Phy in PA resistance is studied by means of the mutants (Kreslavski *et al.* 2013a, 2016; Rusaczonok *et al.* 2015, Khudyakova *et al.* 2017). Thus, some studies demonstrated that increase of PhyB content in transgenic potato plants, Dara-5 and Dara-12, superproducing PhyB, led to higher resistance of PA to high irradiance (Thiele *et al.* 1999) and UV-B (Kreslavski *et al.* 2015) compared to the WT potato. Deficit of PhyB led to reduced resistance of the PA to UV-A in *A. thaliana* plants (Kreslavski *et al.* 2016). We also found that deficit of both PhyA and PhyB had considerable influence on the photochemical activity and chloroplast structure in leaves of plants grown under RL conditions when cryptochromes absorbing approximately up to 500 nm are inactive (Kreslavski *et al.* 2017).

Thus, Phy system can be important not only for formation of chloroplasts from etioplasts but also for supporting normal photosynthesis in processes of growth and development of green plants.

However, it would be good to deepen the understanding how the PA tolerance is linked to pro- or antioxidant balance, chloroplast structure, and light-induced genes activity. For this aim in the present study we investigated how deficit of PhyA and PhyB (*PhyA*, *PhyB* mutant) in *A. thaliana* leaves exposed to UV-B (3.6 and 7.2 kJ m⁻²) affected resistance of PSII to UV-B, chloroplast structure, and H₂O₂ formation under different growth conditions (white light and red light) with more emphasis on white light experiments. We also compared resistance of PA to UV-B in the DMut and *hy2* mutant, which has phytochrome chromophore deficiency due to inhibition of its biosynthesis.

Materials and methods

Plant material and growth conditions: *Arabidopsis thaliana* (ecotype Columbia-0) 26-d-old wild type and mutant (deficient in phytochromes) plants were grown under controlled conditions. *Phytochrome A*, *phytochrome B*-deficient mutant (DMut) having a reduced content of apoproteins of both phytochromes A and B and the *hy2* mutant (long hypocotyl mutant) plants of *A. thaliana* defective in phytochrome chromophore biosynthesis (Parks and Quail 1991) were received from the European Arabidopsis Stock Centre (Nottingham, UK).

All plants were grown in a growth chamber at 12-h photoperiod, day temperature of 24 ± 0.5°C, and 12 h in the dark (21 ± 0.5°C) for 26 d. White fluorescent lamps [130 μmol (photon) m⁻² s⁻¹] or red LEDs [656 nm, 19 nm FWHM, 130 μmol(quantum) m⁻² s⁻¹] were used for growing the plants.

UV-B radiation was provided with narrow-band ultraviolet lamp *PL-S 9W/01/2P* (*Philips Lighting*, Poland) with λ_{max} = 311 ± 3 nm and irradiance = 1 W m⁻² on the surface of irradiated leaves. Recovery of leaves after UV-exposure was provided by white fluorescent tubes [PAR of I = 25 μmol(quantum) m⁻² s⁻¹].

For each variant and any type of plants (WT, DMut, *hy2*) three healthy, developed, upper leaves with almost horizontal position of the leaf blade were chosen from three–four pots for fluorescence measurements. Main experiments were repeated 3–5 times (*n*) using 50–70 plants for each experiment. The leaves were detached and then kept in the dark for 20 min until the measurements. For pigment and growth measurements, 12–15 leaves per each variant were used.

Fluorescence measurements: The values F_0 , F_v , F_m , F_m' , F' , as well as the PSII maximum photochemical quantum yield (F_v/F_m) and the PSII effective photochemical quantum yield $Y(II)$, equal to $(F_m' - F_i)/F_m'$, were determined with PAM fluorometer (*Junior-PAM*, Heinz Walz, Germany). Here, F_m and F_m' are the maximum chlorophyll (Chl) fluorescence levels under dark- and light-adapted conditions. Respectively, F_i is the level of Chl fluorescence before a saturation impulse is applied. F_0 is initial Chl fluorescence level (or Chl fluorescence level of open PSII RC). Actinic light was switched on for 10 min [$I = 190 \mu\text{mol}(\text{quantum}) \text{m}^{-2} \text{s}^{-1}$].

Chl fluorescence induction curves (OJIP curves) were recorded with the help of home-made set-up, which was described earlier (Lankin *et al.* 2014). The JIP-test is often used for evaluation of PSII state (Strasser and Strasser 1995, Strasser *et al.* 2000, Kalaji *et al.* 2012, 2014, Goltsev *et al.* 2016). OJIP curves were measured under illumination with blue light with intensity of $5,000 \mu\text{mol}(\text{quantum}) \text{m}^{-2} \text{s}^{-1}$ for 1 s.

The following fluorescence parameters were calculated on the basis of the fast Chl *a* fluorescence curves: ABS/RC, DI_0/RC , TR_0/RC . Here, ABS/RC ratio averaged absorbed by PSII antenna Chl flux of photons per photochemically active RC, which is determined by the ratio of active/inactive RCs. TR_0/RC is the initial exciton flux captured by one active RC of PSII. $DI_0/RC = (ABS/RC) - (TR_0/RC)$ (total energy dissipated by a PSII RC). $ABS/RC = (M_0/V_j)/(F_v/F_m)$; $TR_0/RC = M_0/V_i$. The fluorescence parameters were calculated by the determination of the following fluorescence values: F_0 , M_0 , V_j , V_i , and F_m . V_j and V_i are fluorescence intensities at time 2 ms (J phase) and 30 ms (I phase) after the start of irradiation by a pulse of actinic light (Fig. 1). M_0 is the value of the initial slope of relative variable Chl fluorescence curve.

The content of Q_B -nonreducing centers was calculated by the method of Klinkovský and Nauš (1994). The formulae $(F_m - F_{pi})/F_v$, where F_{pi} is the fluorescence intensity on the plateau region was used for calculation. Here, plateau is located in the region of the transition from the exponential fluorescence dependence to the sigmoid one.

Pigments: The content of photosynthetic pigments [$\mu\text{g g}^{-1}(\text{FM})$] was calculated on the basis of measurements of the optical density of filtrated ethanol extracts (Lichtenthaler and Wellburn 1987).

UV-absorbing methanol extracted compounds (predominantly flavonoids) (UAPs) were isolated from cuttings of fresh leaves according to Mirecki and Teramura (1984). The 15–25 cuttings from the different leaves were incubated in acidic methanol for 24 h (methanol:water:HCl ratio of 78:20:2) under 4°C. Then the optical density of the solution at 327 nm was determined by the spectrophotometer *Specord M-40* (Karl Zeiss, Jena, Germany). The amount of UV-absorbing compounds (optical density unit per leaf fresh mass) was calculated from these values.

Electron microscopy: For electron microscopy studies, leaf sections from the middle part of the leaf were cut, fixed with 2% glutaraldehyde in phosphate buffer with or without postfixation by 1% osmium tetroxide. Then, after dehydration, the samples were incubated in a number of solutions with increasing alcohol and acetone concentration and embedded in *Epon* epoxide resin. Ultrathin sections were cut by *LKB* ultramicrotome (Sweden) contrasted with uranyl acetate and lead citrate. After this samples were studied with an electron microscope *JEM 100B* (JEOL, Tokyo, Japan) (Semenova and Romanova 2011) and photographed.

Hydrogen peroxide content was measured by luminol-dependent chemiluminescence method (Cormier and Prichard 1968) in leaf homogenates. with chemiluminometer *LUM-100* (*DIsoft*, Russia).

RNA isolation, cDNA synthesis, and Real-Time quantitative PCR: Total RNA was isolated by *TRI-Reagent* (*MRC, Inc.*) using the manufacturer's instructions according to Kreslavski *et al.* (2013a). Plant tissue was destructed in *TRI-Reagent* and chloroform was added for separation of the aqueous and the organic phases. RNA was precipitated from the aqueous phase by isopropanol. The precipitate was washed with 70% ethanol and then it was dissolved in diethyl-treated, autoclaved, distilled water. For the first-strand cDNA synthesis, we used a reaction kit for reverse transcription (*Synthol*, Russia) according to the manufacturer's instructions. A real-time quantitative polymerase chain reaction (qPCR) was provided using *iCycler IQ5* (*Bio-Rad*, USA) and reaction mixtures from the *qPCRmix-HS SYBR* kit (*Evrogen JSC*, Russia).

The *UBQ5* (AT3G62250) gene was used as an internal reference (Gutierrez *et al.* 2008).

The *Oligo* software was used for primer design. Degenerated primers were used for grope of *PAL* genes. The primer sequences were:

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gacgcttcactctgctcc (direct)
ccacaggttgcttag (reverse) for AT3G62250 (UBQ5)
gataggtctgcttcgaaggt (direct)
atgtggcctcagcgtaatca (reverse) for AT1G07890 (APX1)
gtacacgaaagaaggacctggagca (direct)
ttcaaggattacgctgtagcccatg (reverse) for AT4G08390 (sAPX)
ctcccagggcatagtcattgaaa (direct)
agtggacaagaggacaaaacg (reverse) for AT1G77490 (tAPX)
agaggccgaggactgtcttac (direct)
ccaatggctcagcaaggttctc (reverse) for AT1G29930 (CAB1)
agcgatcctagaccaggtgga (direct)
actccaacctctcctctcgt (reverse) for AT5G13930 (CHS)
gggtctcacagtgaacagcga (direct)
ctcttcagcaa accgcactaa (reverse) for AT2G32950 (COPI)
tgcaagagggaaacagagcttgagg (direct)
gctaatgcctttgctgctaaa (reverse) for AT5G54190 (POR-A)
aaatgtggtgctactggagcct (direct)
acgctgtccaacgaggctaaagtc (reverse) for AT4G27440 (POR-B)
ccgagaaggcagcagatctgtt (direct)
aaagtgaccagatggtgttcc (reverse) for AT1G03630 (POR-C)
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agttccgtctgggatg (direct)
 taaaaaggagccgccgaat (reverse) for ATCG00020 (*psbA*)
 gcaagcaagagagagaaaaagg (direct)
 cagcattagaaccaccacc (reverse) for AT5G11260 (*HY5*)
 gcaacaagcaagagagagaa (direct)
 ttgtcatcagtttagccttg (reverse) for AT3G17609 (*HYH*)
 ggctttgtctctgctttct (direct)
 gtgcctaactcatccacccc (reverse) for AT3G09150 (*HY2*)
 acccggtRatgcagaaRctgagaca (direct)
 agctccgttccaactcBttgagaca (reverse) for *PAL*

Results

After growing plants in white light no noticeable difference was revealed in fluorescence induction curves (Fig. 1) and all fluorescence parameters determined in WT, DMut, and *hy2* plants (Table 1). There was also no significant difference in chloroplast structure (Fig. 2). However, we observed slight diminishing in biomass of leaves in DMut and *hy2* compared with WT. Thus, average leaf fresh mass was 13.5 ± 1 , 10.3 ± 0.5 , and 9.3 ± 0.4 mg for WT, DMut, and *hy2*, respectively. DMut leaves looked paler than the WT leaves. The content of Chl and carotenoids was smaller in DMut by about 15–20% compared with WT (Table 2). The UAPs content in the mutants was 1.5 times lower than that in the WT.

Effects of UV-B on white light-grown plants. The UV-B treatment led to significant alternations of parameters reflecting PSII activity (Fig. 1) of WT and DMut samples grown under WL and slight changes in chloroplast structure of WT and DMut plants. Decreasing in all fluorescence parameters was significant already after 1 h of UV-exposure. Wherein, the changes in all fluorescence values were bigger in DMut and *hy2* compared with WT.

After UV exposure, increase in DI_0/RC and ABS/RC values and the content of Q_B -nonreducing centers of PSII (Q_B -NC) in samples grown under WL was bigger for *hy2* and DMut compared with WT (Table 1). For example, value of Q_B -NC increased after 1-h UV-exposure from 35% up to 55% for WT and from 36% up to 81% for DMut. A reliable difference in decrease of effective quantum yields between WT and *hy2* or DMut variants was observed after 1-h UV exposure and after 2-h UV exposure for DMut. A reliable difference in decrease of maximal quantum yield between WT and DMut or *hy2* was observed for all variant, excluding *Hy2* (2 h) variant. Here, considerable reductions in quantum yields after 1-h UV exposure were not accompanied with reliable increase in H_2O_2 content. However, the reliable increase in H_2O_2 content in WT and DMut samples after 2-h UV treatment was detected. In this case, in WT samples the content of H_2O_2 increased from 1.65 ± 0.12 to $2.3 \pm 0.09 \mu\text{mol g}^{-1}$ (FM) and in DMut samples, it increased from 1.35 ± 0.08 to $2.43 \pm 0.06 \mu\text{mol g}^{-1}$ (FM).

here *R* – a/g and *B* – c/t/g. *PAL*-genes (degenerated primers for four genes) – AT2G37040 (*PAL1*), AT3G53260 (*PAL2*), AT5G04230 (*PAL3*), and AT3G10340 (*PAL4*).

Statistics: The tables and figures show the arithmetic averages of the obtained values and the corresponding standard error (\pm SE). Presented values are of three, four or five biological and four–seven analytical replications. The significance of differences between any two variants (experiment and control) was described by *Student's t*-test at the 5% significance level.

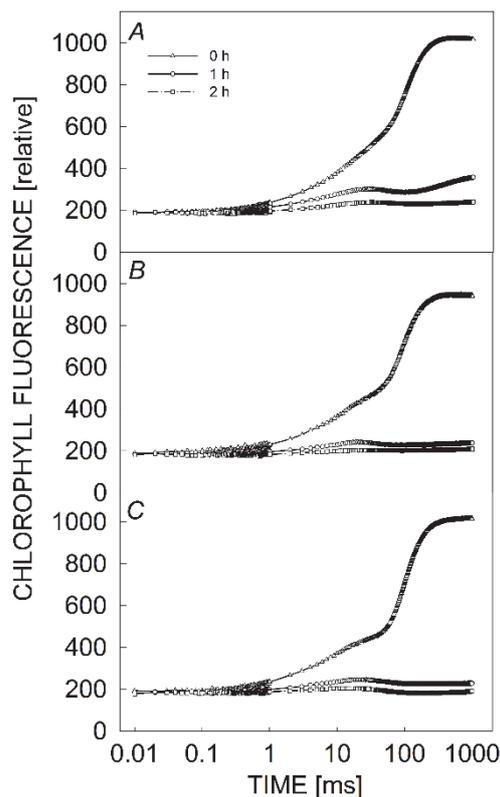


Fig. 1. Chlorophyll *a* fluorescence induction curves of wild-type (A), *phyA phyB* double mutant (B), and *hy2* mutant (C) of *Arabidopsis thaliana* plants grown under white light. The detached leaves were exposed to UV-B radiation for 1 h (1 h, circles) and 2 h (2 h, rectangles) or not exposed (0 h, triangles). Typical curves are shown.

In all cases, we observed partial light-induced recovery in detached leaves after 1-h and 2-h UV-exposure that did not depend on type of plants – WT or DMut. It was small and reliable already 0.5 h after finishing 1-h UV exposure and more significant after 3-h irradiation with WL (Table 1). The recovery was stopped in the dark with protein inhibitor chloramphenicol (3 mM). Note that recovery in native leaves, which were irradiated with UV-B for 1 h was more effective than the recovery of detached leaves.

Table 1. The effect of phytochrome deficiency and UV-B radiation on fluorescence parameters of 26-d-old *Arabidopsis thaliana* WT, DMut and *hy2* plants grown in white light ($I = 130 \mu\text{mol}(\text{quantum}) \text{m}^{-2} \text{s}^{-1}$ PAR, photoperiod 12 h). The leaves were detached and kept for 0.5 h in dark. Then they were exposed or not exposed (control) to UV-B treatments (1 h, 2 h) and recovered for 3 h under white light ($F_v/F_m \text{ rec}$). $Q_B\text{-NC}$ – the amount of Q_B -non-reducing centers of PSII, % of total amount of the centers. N – the number of leaves. Leaf fresh biomass was 13.5 ± 1 , 10.3 ± 0.5 , and 9.3 ± 0.4 for WT, DMut and *hy2*, respectively. The amount of leaves per a plant was 10 ± 1 for WT and 9 ± 1 for DMut and *hy2*. Means \pm SE of approximately 30–40 leaves, $n = 4$. * – difference between WT under UV and *hy2* or DMut under UV is insignificant, # – determination was hampered.

Parameter/variant	F_v/F_m	$F_v/F_m \text{ rec}$	Y(II)	$Q_B\text{-NC}$ [%]	DI_0/RC	ABS/RC
WT	0.80 ± 0.01	-	0.46 ± 0.01	34 ± 3	0.23 ± 0.02	1.3 ± 0.1
DMut	0.80 ± 0.01	-	0.44 ± 0.03	36 ± 2	0.25 ± 0.03	1.3 ± 0.1
<i>Hy2</i>	0.79 ± 0.01	-	0.39 ± 0.02	32 ± 3	0.27 ± 0.06	1.5 ± 0.1
WT(1 h)	0.40 ± 0.02	0.64 ± 0.03	0.29 ± 0.03	55 ± 3	1.46 ± 0.25	2.6 ± 0.2
DMut(1 h)	0.34 ± 0.03	$0.55 \pm 0.04^*$	$0.23 \pm 0.02^*$	81 ± 5	3.4 ± 0.3	$3.2 \pm 0.2^*$
<i>Hy2</i> (1 h)	0.30 ± 0.02	0.48 ± 0.03	0.19 ± 0.01	#	3.1 ± 0.4	5.3 ± 0.4
WT(2 h)	0.22 ± 0.15	0.47 ± 0.02	0.14 ± 0.02	#	4.8 ± 0.3	5.9 ± 0.4
DMut(2 h)	0.15 ± 0.02	0.31 ± 0.02	$0.09 \pm 0.01^*$	#	8.6 ± 0.7	8.6 ± 0.4
<i>Hy2</i> (2 h)	$0.18 \pm 0.02^*$	0.28 ± 0.03	$0.11 \pm 0.03^*$	#	6.8 ± 0.5	7.3 ± 0.5

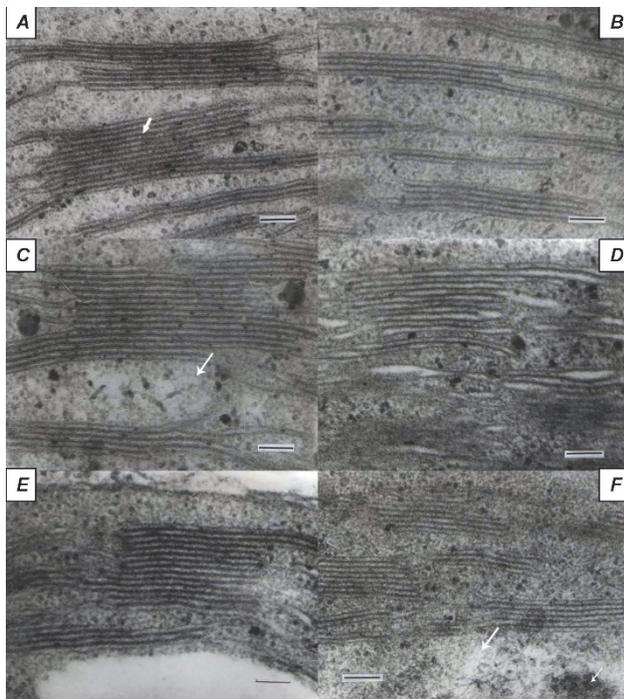


Fig. 2. Organization of thylakoids in grana chloroplasts of *Arabidopsis thaliana* WT (A,C,E) and DMut (B,D,F) plants grown under white light and exposed to UV-radiation (C,E,D,F) and then recovered for 3 h under white light (E,F) or non-exposed (A, B) to UV radiation. All conditions are described in the section “Materials and methods”. Arrows on panels C,F show nucleoid-like structures, in which one can see core (thin *small arrow* on panel F). Arrow shows the electron dense contact strip between thylakoids (A). Bars on micrograph are equal to 100 nm.

For example, the F_v/F_m ratio after 1-h exposure of WT native leaves was 0.53 ± 0.03 , after 5-h recovery under WL [$15 \mu\text{mol}(\text{quantum}) \text{m}^{-2} \text{s}^{-1}$ PAR], the value of F_v/F_m was higher than that in detached recovered leaves and reached

0.71 ± 0.04 . Such effect can indicate that PSII damage is mainly linked to inactivation of D1 protein, which is easily damaged and then is quickly synthesized (Andersson and Aro 2001).

Effects of UV-B in red light-grown plants: WT and DMut plants grown under red light showed more significant differences in shape of induction curves (Fig. 3) as well as all fluorescence parameters (Table 3) after UV-treatment. Quantum yields [F_v/F_m , Y(II)] also declined more in DMut compared with WT, whereas other fluorescence parameters, such as DI_0/RC (total energy dissipated in PSII), ABS/RC ratio, and the content of Q_B -nonreducing centers, increased.

Here, difference effect between fluorescence parameters determined in WT and DMut plants grown under RL (Table 1) was much bigger than the same effect in plants grown under WL (Table 3). Moreover, UV affected the PSII activity stronger in DMut samples grown under RL than in DMut samples grown under WL, whereas for WT samples such effect was absent (Figs. 1, 3).

Chloroplast structure changes: UV radiation led to some changes in the structure of thylakoid membranes (Fig. 2A–F). Usually we did not observe a difference in amount of thylakoids within the grana stacks, but in some chloroplasts, the average number of thylakoids within the grana stacks of DMut was lower than that in the WT. We observed only a slight swelling of the WT and DMut thylakoids and a strong swelling in the marginal thylakoids of DMut (Fig. 2D), which was no longer manifested after restoration (Fig. 2F). After UV irradiation, the nucleoid-like structures were visualized both in the WT and in the DMut (the oval areas marked with arrows on Fig. 2C,F), which initially were not observed before irradiation. These structures were also conserved after the restoration of PSII activity (Fig. 2F).

Table 2. The photosynthetic and UV-absorbing pigments (UAPs) content in leaves of 26-d-old *Arabidopsis thaliana* WT and DMut plants grown in white and red light with intensity of $130 \mu\text{mol}(\text{quantum}) \text{m}^{-2} \text{s}^{-1}$ PAR, photoperiod 12 h. Data on UAPs are given in relative units arbitrary accepting content of UAPs in DMut-RL for 1. For the comparison, the data for *hy2* grown under the same conditions in WL are given, $n = 3$. * - difference between WT and DMut or *hy2* is unreliable ($p > 0.05$).

Variant	Chl (<i>a+b</i>) [$\mu\text{g g}^{-1}(\text{FM})$]	Car [$\mu\text{g g}^{-1}(\text{FM})$]	UAPs [rel. units]
WT(WL)	662 ± 29	112 ± 7	1.7 ± 0.2
DMut(WL)	539 ± 31	$95 \pm 7^*$	1.15 ± 0.10
Hy2(WL)	528 ± 26	$92 \pm 8^*$	1.04 ± 0.12
WT(RL)	495 ± 24	91 ± 6	2.80 ± 0.35
DMut(RL)	310 ± 35	55 ± 7	1.0 ± 0.2

Transcriptional levels of genes. Our study provided comparison of the transcriptional levels of some genes encoding antioxidant enzymes, such as ascorbate peroxidase 1, thylakoid-bound ascorbate peroxidase (tAPX), stromal ascorbate peroxidase (sAPX), chalcone synthase (CHS), phenylalanine ammonia-lyase (PAL), photosynthetic proteins: D1 and chlorophyll *a/b* binding

protein (CAB1) as well as enzyme of Chl-biosynthesis, protochlorophyllid-oxidoreductase (Table 4). The transcriptional levels of the *POR-B*, *POR-C*, and *sAPX* genes of plants, grown under WL, were lower in the DMut compared with the WT. In plants grown under RL, the transcriptional level of the *hy2* gene was lower in the DMut compared with the WT.

Discussion

The role of various phytochromes in the processes of growth and photomorphogenesis has been largely studied (Whitelam and Devlin 1997, Park and Song 2003, Carvalho *et al.* 2016, Kaiserli and Chory 2016). It is also known that different types of phytochrome are involved in the mechanisms of adaptation to environmental stress factors (Carvalho *et al.* 2011, Markovskaya *et al.* 2016, Rusaczonok *et al.* 2015). On the other hand, little is known about the relationship between the phytochrome system and the photosynthetic processes under stress.

The PA plays an important role in the development of mechanisms for adaptation and plant resistance to environmental stressful factors, therefore, it is important to understand, how photoreceptors such as phytochromes regulate processes of PA adaptation and its stress resistance (Allakhverdiev *et al.* 1997, 2007; Shapiguzov *et al.* 2005, Kreslavski *et al.* 2007, Mohanty *et al.* 2007, Murata *et al.* 2007, Allakhverdiev 2011).

A number of studies demonstrated that an increase in the content of PhyB or its active form has little or no effect on the photosynthetic parameters under the normal conditions but increases the content of photosynthetic pigments and UAPs as well as the resistance of PA to UV radiation or other stressful factors (Joshi *et al.* 1991, Lingakumar and Kulandaivelu 1993, Biswal *et al.* 1997, Thieli *et al.* 1999, Foreman *et al.* 2011, Rao *et al.* 2011, Kreslavski *et al.* 2012, 2013a,b, 2015, 2016; Rusaczonok *et al.* 2016). However, the specific mechanisms of the action of Phy are largely unclear. Therefore, many researchers are focused at studying of the Phy system role in the mechanisms of resistance of plants and their PA to the action of abiotic environmental stressors. However, PhyB often acts together with PhyA (Casal 2000, Sineshchekov 2010). Therefore, our effort was focused on the study of PSII photochemical activity in plants deficient

in PhyB and PhyA under stressful conditions induced with UV-B irradiation of several doses. Doses of 3.6 and 7.2 kJ m^{-2} used in our study are moderate and can induce both damaging and photomorphogenetic effects in plants. Here, different responses to UV-B, including gene regulation and UV-B tolerance, can be induced by photoreceptor UVR8 (Jenkins 2014).

It is known that UV-B can affect corresponding molecular targets either directly or by ROS which are generated during UV-irradiation. Degradation of PSII key proteins, such as D1 and D2, and photosynthetic pigments,

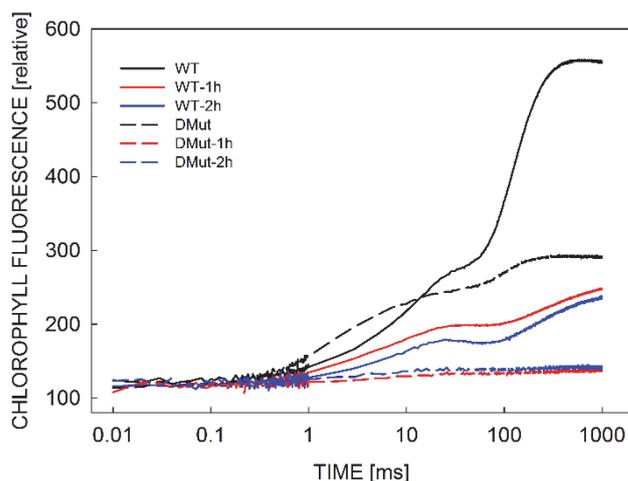


Fig. 3. Chlorophyll fluorescence induction curves of wild type (WT, continuous lines) and *phyA phyB* double mutant (DMut, dashed lines) of *Arabidopsis thaliana* plants grown under red light. Detached leaves were irradiated with UV-B for 1 h (WT-1h and DMut-1h, red lines) and 2 h (WT-2h and DMut-2h, blue lines) or not irradiated (WT and DMut, black lines). Typical curves are shown.

Table 3. The effect of phytochrome deficiency and UV-radiation dose on fluorescence parameters of 26-d-old *Arabidopsis thaliana* WT and DMut plants grown in red light ($I = 130 \mu\text{mol}(\text{quantum}) \text{m}^{-2} \text{s}^{-1}$, photoperiod 12 h). The leaves were detached and kept for 0.5 h in dark. Then they were exposed to UV-B treatments (1 and 2 h). $Q_B\text{-NC}$ – the amount of Q_B -non-reducing centers of PSII. Leaf fresh mass was 16.2 ± 1.3 and 3.3 ± 0.8 mg for WT and DMut, respectively. N – the number of leaves, $n = 4$. All differences between WT and DMut are reliable ($p < 0.05$), # – determination was hampered.

Variant	F_v/F_m	Y(II)	DI_0/RC	$Q_B\text{-NC}$ [%]	ABS/RC	N
WT	0.78 ± 0.01	0.34 ± 0.02	0.27 ± 0.02	32 ± 4	1.2 ± 0.1	9 ± 1
DMut	0.64 ± 0.03	0.25 ± 0.04	0.53 ± 0.04	54 ± 5	1.7 ± 0.1	8 ± 1
WT (1 h)	0.53 ± 0.01	0.22 ± 0.02	0.88 ± 0.08	56 ± 4	1.9 ± 0.1	-
DMut (1 h)	0.37 ± 0.04	0.16 ± 0.01	2.3 ± 0.2	80 ± 7	3.5 ± 0.1	-
WT (2 h)	0.49 ± 0.03	0.19 ± 0.01	1.34 ± 0.09	#	2.6 ± 0.2	-
DMut (2 h)	0.21 ± 0.02	0.13 ± 0.02	3.4 ± 0.4	#	4.2 ± 0.2	-

Table 4. The transcriptional activity of some antioxidant and photosynthetic genes in the 26-d-old *Arabidopsis* leaves of the WT and mutants DMut, grown under WL (WT1 and DMut1) and red light (WT2 and DMut2). Activities of WT grown under WL are normalized to 1. Means \pm SE, $n = 5$. * – reliable differences between WT1 and DMut1 or WT2, $p < 0.05$. ** – reliable differences between WT2 and DMut2, $p < 0.05$.

Gene	Protein	WT1	DMut1	WT2	DMut2
<i>AT1G07890</i>	APX1	1.0 ± 0.2	0.66 ± 0.17	0.95 ± 0.16	0.62 ± 0.50
<i>AT4G08390</i>	sAPX	1.0 ± 0.1	$0.44 \pm 0.12^*$	$0.53 \pm 0.13^*$	0.85 ± 0.15
<i>AT1G77490</i>	tAPX	1.0 ± 0.3	1.30 ± 0.25	1.30 ± 0.30	1.20 ± 0.30
<i>AT5G13930</i>	CHS	1.0 ± 0.1	0.75 ± 0.20	$0.55 \pm 0.08^*$	1.13 ± 0.40
-	PAL	1.0 ± 0.2	1.00 ± 0.10	1.30 ± 0.15	1.80 ± 0.20
<i>AT1G29930</i>	CAB1	1.0 ± 0.2	1.50 ± 0.20	0.57 ± 0.14	0.40 ± 0.15
<i>AT5G54190</i>	POR-A	1.0 ± 0.1	0.80 ± 0.07	1.10 ± 0.10	1.90 ± 0.40
<i>AT4G27440</i>	POR-B	1.0 ± 0.2	$0.40 \pm 0.05^*$	1.00 ± 0.09	2.00 ± 0.80
<i>AT1G03630</i>	POR-C	1.0 ± 0.2	$0.25 \pm 0.10^*$	0.74 ± 0.13	1.00 ± 0.40
<i>AT3G17609</i>	HYH	1.0 ± 0.2	0.80 ± 0.20	1.90 ± 0.30	$3.80 \pm 0.50^{**}$
<i>AT5G11260</i>	HY5	1.0 ± 0.3	1.50 ± 0.35	1.20 ± 0.20	0.60 ± 0.20
<i>AT3G09150</i>	HY2	1.0 ± 0.2	1.70 ± 0.40	0.70 ± 0.12	$0.20 \pm 0.1^{**}$

release of endogenous Mn-ions from PSII Mn-containing water-oxidizing complex, loss of Rubisco activity, and damage of thylakoid membranes are the main scenarios of UV-induced effects (Kataria *et al.* 2014). Besides, UV-B may modify chloroplast DNAs either directly or by ROS generation (Rastogi *et al.* 2010). Likely, such effects take place under UV-B in our case since we observed changes in DNA filaments as it can be seen from electron microscope pictures (Fig. 2).

Plastid DNA with different proteins and RNA is located in the nucleoid, which is subjected to dynamic changes during the development of plant cells (Sato *et al.* 2003). Apparently, chloroplast DNA is normally dispersed throughout the chloroplast in the stroma, and under the action of stress (UV) it is collected into so-called nucleoids that are well identified and in which the chloroplast DNA filaments condense into dense conglomerates (Fig. 2C,F).

PSII activity in WT plants grown under WL was the same as the PSII activity in DMut or *hy2* plants. However, the PSII activity in DMut plants grown under RL was smaller than the one in the WT plants (Table 3). Besides, the PSII activity in WT or DMut plants grown in RL was smaller than the corresponding activity of plants grown under WL (Tables 1, 3). The reduction in the PSII activity

was expressed as reduction in quantum yields, F_v/F_m and Y(II), as well as increase in amount of Q_B -non-reducing complexes, reducing plastoquinone pool size, and increase in rate of light energy dissipation predominantly into heat (Tables 1, 3). One of the reasons that reduce PSII activity can be some destroying of the thylakoid membrane structure of DMut compared with WT in plants grown under RL. Such effect was demonstrated in previous study in DMut and WT of *A. thaliana* plants (Kreslavski *et al.* 2017) and can be a sequence of low content of photosynthetic pigments in DMut leaves compared to WT samples (Table 2).

Moreover, the significant UV-induced effect of increase in amount of Q_B -nonreducing PSII centers was found. We suggest that the increase in Q_B -nonreducing PSII centers is linked to accumulation of functionally inactive PSII complexes due to the slowdown of D1 protein biosynthesis (Larocca *et al.* 1996). Observed fast PSII recovery, especially significant in native leaves, is also coordinated with a role of inactivation of D1 and D2 proteins, which are easily damaged and then is quickly synthesized (Andersson and Aro 2001) and inserted in PSII complex.

The question arises, how is possible to explain the

difference between WT and DMut plants grown under RL.

The reduced activity of PSII in DMut (RL) compared with WT and in RL plants compared to WL ones occurs likely due to lower content of UV-B-absorbing pigments, such as carotenoids and flavonoids. Indeed, we found that contents of carotenoids and UAPs in DMut was lower than that in WT (Table 2). The decreased Chl content may be due to reduced expression levels of genes encoding POR proteins in DMut compared to WT-grown under WL (Table 4). One can think that smaller content of UAPs and carotenoids in the DMut may be the one of the main reasons of stronger damaging effects of UV-B on PA in DM. Especially significant difference between DMut and WT in pigment content was revealed in RL-grown plants, in which this difference was bigger than in WL-grown plants. Another reason of reduced PSII resistance may be decreased transcriptional activity of genes encoding some antioxidant enzymes (sAPX and CHS) or enzymes such as *hy2* (phyto-

chromobilin synthase) in DMut compared with WT.

In principle, the stronger damage of PSII in DMut or *hy2* compared to WT can be linked to stronger damage of the DMut or *hy2* PSII under UV or the deterioration of PSII recovery in the mutants compared to the WT. However, we did not observe a significant difference between recovery in WT and DMut or *hy2* (Table 1). It is suggested that a damage to PSII is sequence of direct inactivation of the proteins by UV-B absorbance and the action of ROS induced by UV-B irradiation. This is consistent to our data on increase in H₂O₂ content after UV-irradiation.

Conclusion: The PSII in *A. thaliana* DMut is more sensitive to moderate doses of UV-B radiation than the PSII of WT. The resistance of the PSII to UV-B radiation depends on content of phytochromes, first of all PhyB and PhyA, and, likely, blue light photoreceptors – cryptochromes.

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