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# Effect of cryptochrome 1 deficiency and spectral composition of light on photosynthetic processes in *A. thaliana* under high-intensity light exposure

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## Abstract

The role of cryptochrome 1 in photosynthetic processes and pro-/antioxidant balance in the *Arabidopsis thaliana* plants was studied. Wild type (WT) and *hy4* mutant deficient in cryptochrome 1 grown for 20 d under red (RL, 660 nm) and blue (BL, 460 nm) light at an RL:BL = 4:1 ratio were kept for 3 d in different lights: RL:BL = 4:1, RL:BL:GL = 4:1:0.3 (GL – green light, 550 nm), and BL, then were exposed to high irradiance (4 h). Activity of PSII and the rate of photosynthesis in WT and *hy4* decreased under the high irradiance in all spectral variants but under BL stronger decrease in the activity was found in the *hy4* mutant than in WT. We assumed that lowered resistance of photosynthetic apparatus in the *hy4* mutant may be associated with the low activity of the main antioxidant enzymes and reduced content of low-molecular-mass antioxidants in the mutant compared to the WT.

*Keywords*: *Arabidopsis thaliana*; cryptochrome; high-intensity light; photosynthesis; pro-/antioxidant balance.

## Introduction

The light spectral composition plays an important role in the regulation of many physiological processes in plants. The effect of light of different spectral composition on metabolic processes, as well as adaptation to changes in light spectrum and intensity, is realized by a known set of cellular photoreceptors, such as red/far-red light receptors – phytochromes and blue/UV-A light receptors – cryptochromes (Kong and Okajima 2016, Voitsekhovskaja 2019). However, a specific green light (GL) photoreceptor has not so far been found (Li *et al.* 2021). It is possible both an indirect effect of light on the photosynthetic apparatus (PA) of plants by regulating photoreceptor-dependent gene expression (Kleine *et al.* 2007, Kreslavski *et al.* 2009, 2018; D'Amico-Damião and Carvalho 2018) and direct effects of red light (RL) or blue light (BL) (Allakhverdiev *et al.* 2016). For example, the damaging

## Highlights

- Content of photosynthetic pigments was the lowest in the *hy4* mutant under blue light
- Cryptochrome 1 deficit enhanced photoinhibition induced by high irradiance
- The most severe decrease in photosynthetic activity showed *hy4* under blue light

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*Abbreviations*: APX – ascorbate peroxidase; BL – blue light; $DI_0/RC$ – quantum yield of energy dissipation; $F_v/F_m$ – PSII maximal quantum yield; GL – green light; GPX – guaiacol-dependent peroxidase; HIL – high-intensity light; PA – photosynthetic apparatus; $PI_{ABS}$ – PSII performance index; RL – red light; TBARS – thiobarbituric acid reactive substances; TEAC – trolox equivalent antioxidant capacity.

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effect of BL on the oxygen-releasing Mn-containing complex PSII has been shown (Takahashi and Badger 2011).

Three cryptochrome genes encoding light-sensitive proteins Cry1, Cry2, and Cry3, have been identified in the genome of *A. thaliana* plants (Liu *et al.* 2011, D'Amico-Damião and Carvalho 2018, Voitsekhovskaja 2019). Cryptochromes 1 and 2 regulate many physiological processes, most notably plant growth and photomorphogenesis, as well as the biosynthesis of many photosynthetic proteins and enzymes, in particular the key enzyme of the Calvin Rubisco cycle (Lin and Todo 2005, Chaves *et al.* 2011, Liu *et al.* 2011, 2016; D'Amico-Damião and Carvalho 2018, Voitsekhovskaja 2019).

Analysis of gene expression in response to high-intensity light (HIL) has revealed a regulatory role for cryptochrome 1 in the response of multiple genes to HIL (Kleine *et al.* 2007). In contrast, cryptochrome 2 is degraded even under low light and is involved in photoperiodism (Lin *et al.* 1998, D'Amico-Damião and Carvalho 2018, Fantini *et al.* 2019). In addition, cryptochrome 1 is an indicator of the BL/GL ratio in the spectrum of incident light in some physiological processes and this was demonstrated by the example of changes in hypocotyl elongation in *Arabidopsis* plants (Sellaro *et al.* 2010, Wang and Folta 2013). This effect of hypocotyl elongation is one of the symptoms of 'shade avoidance syndrome' (Smith and Whitelam 1997, Sellaro *et al*. 2010), which is caused by the fact that the plants of the lower tiers are illuminated by an altered solar spectrum, devoid of the red and blue range, which are noticeably absorbed by the leaves of upper tiers.

BL and GL are known to regulate development and growth *via* photoreceptors, such as phytochromes and cryptochromes (Folta and Maruhnich 2007). However, little information is available on the photosynthesis and antioxidant status of plants under different BL/GL ratios in the irradiance spectrum, which affects, as we hypothesized, the activity of cryptochrome 1 in the regulation of photosynthetic processes and pro-/antioxidant balance (Kreslavski *et al.* 2023). However, the role of cryptochromes in the light regulation of these processes under both stress and physiological conditions is still poorly understood, in particular the interaction of GL with cryptochrome 1 in the defense of PA against HIL and other stress factors.

There is growing evidence that cryptochromes, which mainly absorb in the UV-A and BL regions, also act as key regulators of several plant stress responses, such as responses to UV-B and high light (D'Amico-Damião and Carvalho 2018, Khudyakova *et al.* 2022). Thus, data on plant responses to abiotic stress that are modulated by cryptochromes were discussed in a recent review (D'Amico-Damião and Carvalho 2018). Cryptochrome 1 is likely to play a particularly important role in the PA response to HIL, which has been tested using mutants deficient in this cryptochrome (Kleine *et al.* 2007, Kreslavski *et al.* 2009, 2020, 2023). In particular, the *hy4* mutant with the deficit in cryptochrome 1 (Ahmad and Cashmore 1993) and lost hypocotyl repression under blue light was used. However, the role of cryptochrome 1 in protecting PA from the negative effects of HIL is so far poorly understood.

In the present work, the role of cryptochrome 1 and GL in photosynthetic reactions and changes in the balance of pro-/antioxidants balance of *A. thaliana* under short-term HIL was studied. For this purpose, the *A. thaliana hy4* mutant and WT were grown for 3 d at different ratios of RL, GL, and BL and after this were exposed to 4-h high irradiance.

## Materials and methods

**Cultivation of plants and scheme of the experiment**: Plants of *A. thaliana* WT (Col-0 ecotype) and *hy4* mutant deficient in cryptochrome 1 (catalog number CS70) were used in experiments. Seeds were obtained from the *Nottingham Arabidopsis Stock Center* (Nottingham, UK). The plants were grown for 20 d under light at RL:BL = 4:1 ratio and at 100 μmol(photon) $m^{-2}$ $s^{-1}$ intensity. Then, one part of the plants was left to grow under the same light conditions and another part was moved under different light with RL:BL:GL = 4:1:0.3 ratio, a third part was transferred to BL [100 μmol(photon) $m^{-2}$ $s^{-1}$], and all plants were grown for 3 d. Then, plants were exposed to HIL from white LEDs [4 h, 1,000 μmol(photon) $m^{-2}$ $s^{-1}$]. All the time, plants grew under a 12-h photoperiod at 22 ± 1°C and 20 ± 1°C at night.

**Photochemical activity**: Fluorescence parameters were estimated based on the JIP test by the fluorimeter described in Kreslavski *et al.* (2014). To determine the minimum ($F_0$) and maximum ($F_m$) of Chl fluorescence, weak measuring BL [$\lambda_m$ = 455 nm, 0.25 μmol(photon) $m^{-2}$ $s^{-1}$] and a saturating BL pulse [$\lambda_m$ = 455 nm, 5,000 μmol(photon) $m^{-2}$ $s^{-1}$, 1 s duration], respectively, were used. Based on the data obtained from OJIP induction curves, the values that characterize the state of PSII were calculated: $F_v/F_m$, $DI_0$/RC, and $PI_{ABS}$ (Stirbet and Govindjee 2011, Goltsev *et al.* 2016). $F_v/F_m$ is the maximum photochemical quantum yield of PSII, where $F_v$ is variable fluorescence defined as the difference between $F_m$ and $F_0$. The value of $DI_0$/RC = (ABS/RC) – ($TR_0$/RC) represents the amount of energy dissipated predominantly into heat by the reaction center of PSII, and $PI_{ABS}$ = (ABS/RC) × ($F_v/F_0$) × [$ET_0$/($TR_0$ – $ET_0$)] is the PSII performance index. ABS/RC is the flux of absorbed energy per active reaction center, $TR_0$/RC is the maximum energy flow absorbed by all PSII reaction centers and used for the primary charge separation in the PSII reaction center, and $ET_0$ is the electron flux from $Q_A$ to $Q_B$.

**$CO_2$ gas exchange and stomatal conductance**: Photosynthetic rates and leaf stomatal conductance were measured by a *LCPro+* portable gas exchange analyzer (*ADC BioScientific Ltd.*, UK) in an open system at a temperature of 21 ± 0.5°C, $CO_2$ concentration of 430 ± 15 μmol $m^{-2}$ $s^{-1}$, and relative humidity of 70–80%. The light intensity of 600 μmol(photon) $m^{-2}$ $s^{-1}$ saturating

for *A. thaliana* plants was used for measuring $CO_2$ gas exchange. Photosynthetic rates and stomatal conductance were recorded for 6–8 min.

**Content of photosynthetic pigments**: For determination content of Chl *a*, *b*, and carotenoids, 0.5 g of fresh leaves were ground in a mortar with 1–2 ml of 96% ethanol with the addition of $MgCl_2$. Then, the homogenate was diluted to 5 ml and centrifuged at 10,000 rpm for 10 min at 4°C (*ELMI CM50*, Riga, Latvia). The absorbance of the supernatant was measured by *Genesys 10 UV* spectrophotometer (*Thermo Fisher Scientific*, USA) at $\lambda_{max}$ – 470, 649, and 665 nm, as described elsewhere (Lichtenthaler 1987). The content of photosynthetic pigment was determined as μg $g^{-1}$(FM).

**Antioxidant enzyme activity and thiobarbituric acid reactive substances**: The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was determined according to the method of Nakano and Asada (1981) through the decrease in absorbance at 290 nm due to the oxidation of ascorbate. In this case, the decrease in absorbance at 290 nm as a result of ascorbate oxidation was determined. Guaiacol-dependent peroxidase (GPX, EC 1.11.1.9) activity was determined according to the method of Balakhnina and Nadezhkina (2017) for the conversion of guaiacol to the oxidized tetra guaiacol form and monitored at 470 nm. The content of TBARS was determined as described by Balakhnina and Nadezhkina (2017). The absorbance of TBARS was measured at 532 and 600 nm using a *Hitachi-557* spectrophotometer (Kyoto, Japan). All results obtained were calculated per 1 g of FM.

**Trolox equivalent antioxidant capacity (TEAC)**: TEAC was evaluated using a *Hitachi-557* spectrophotometer (Kyoto, Japan) by the method described in Re *et al.* (1999) and involved reaction of methanolic extracts with the 2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt (ABTS) (*Sigma-Aldrich*, Burlington, MA, USA, CAS no. 30931-67-0). The value of TEAC was expressed as μmol(Trolox) $g^{-1}$(FM).

**Statistics**: Three–six biological and at least 6–10 analytical replicates were used for each experiment. One-factor analysis of variance (*ANOVA*) using *SigmaPlot 12.3* software (*Systat Software Inc.*, San Jose, CA, USA) was used. Different letters were used to indicate significant differences between WT and the *hy4* mutant at $p<0.05$. The values presented in the tables and figures are the arithmetic means ± SD.

## Results

**Plant morphological features**: The leaf area of WT and *hy4* mutant was maximal in plants at the RL:BL:GL = 4:1:0.3 ratio, minimal at BL, and the intermediate value was in the RL:BL = 4:1. At the same time, the leaf fresh mass of the mutant was less than that of WT in RL:BL:GL = 4:1:0.3 option by about 10–15%, in the RL:BL, it was 20–25%, and the largest difference between WT and *hy4* was in BL variant (30–35%). Also on BL, the *hy4* mutant had a longer hypocotyl compared to WT and compared to other spectral options. The most upright leaf position was observed in the *hy4* mutant under BL. Irradiation with HIL for 4 h had an insignificant effect on these morphological parameters of plants.

**Photosynthetic activity**: Initially, the PSII photochemical activity did not differ much among the different spectral options. Thus, PSII activity (expressed as $PI_{ABS}$ and $F_v/F_m$) in WT was around 4–5 for $PI_{ABS}$ and 0.79–0.80 for $F_v/F_m$ regardless of the spectral distribution at plant illumination (Fig. 1*A*,*C*). The activity of PSII in *hy4* was also nearly identical, ranging between 3 and 4 for $PI_{ABS}$ and 0.77 and 0.79 for $F_v/F_m$ regardless of spectral distribution when plants were grown. At BL, the photosynthetic rate ($P_N$) in WT and *hy4* was maximum, while the rate in WT was higher than that in *hy4* (Fig. 2). In other lighting options, the difference was minimal. Stomatal conductance ($g_s$) in both WT and the *hy4* mutant in the RL:BL and WT in BL option differed little, but at the BL option, the $g_s$ of the mutant was lower compared to WT (Table 1).

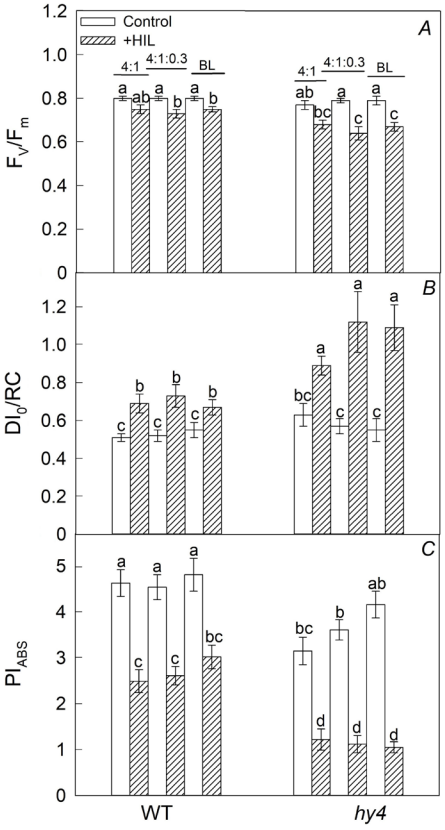


Fig. 1. Effect of high-intensity light (HIL) [4 h, 1,000 μmol(photon) $m^{-2}$ $s^{-1}$] on fluorescence parameters $F_v/F_m$ (*A*), $DI_0/RC$ (*B*), and $PI_{ABS}$ (*C*) in WT and *hy4* plants grown under LEDs of different spectral compositions: RL:BL = 4:1, RL:BL:GL = 4:1:0.3 and BL at a light intensity of 100 μmol(photon) $m^{-2}$ $s^{-1}$. Mean values ± SD are shown. *Different letters* correspond to significant differences in values at $p<0.05$, $n = 6$. $F_v/F_m$ – PSII maximal quantum yield; $DI_0/RC$ – quantum yield of energy dissipation; $PI_{ABS}$ – PSII performance index.

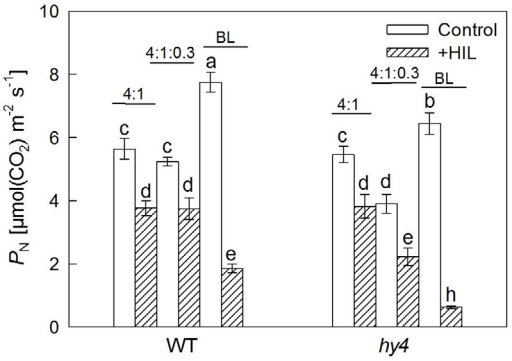


Fig. 2. Effect of high-intensity light (HIL) on the photosynthetic rate ($P_N$) in WT and *hy4* plants. Mean values ± SD are shown. *Different letters* correspond to significant differences in values at $p<0.05$, $n = 4$.

Table 1. Effect of high-intensity light (HIL) on stomatal conductance ($g_s$) in WT and *hy4* plants grown under LEDs of two spectral compositions: RL:BL = 4:1 and BL at a light intensity of 100 μmol(photon) $m^{-2}$ $s^{-1}$. Mean values ± SD are shown. *Different letters* correspond to significant differences in values at $p<0.05$, $n = 6$.

| Options/parameters | | $g_s$ [mmol $m^{-2}$ $s^{-1}$] |
|---|---|---|
| RL:BL = 4:1 | WT | 80 ± 5$^{c}$ |
| | WT+HIL | 111 ± 4$^{a}$ |
| | *hy4* | 72 ± 4$^{cd}$ |
| | *hy4*+HIL | 99 ± 2$^{b}$ |
| BL | WT | 81 ± 3$^{c}$ |
| | WT+HIL | 79 ± 3$^{c}$ |
| | *hy4* | 68 ± 2$^{d}$ |
| | *hy4*+HIL | 65 ± 4$^{d}$ |

The action of HIL led to a decrease in the activity of PSII ($F_v/F_m$ and $PI_{ABS}$ values) (Fig. 1*A*,*C*) and photosynthetic rate ($P_N$) (Fig. 2) in all variants. At the same time, the decrease in photosynthetic rate under HIL action [4 h, 1,000 μmol(photon) $m^{-2}$ $s^{-1}$] was approximately the same in the *hy4* mutant and WT in the RL:BL (4:1) and RL:BL:GL options. However, the BL spectral variant showed a particularly marked difference – the decrease of $P_N$ in *hy4* was 10-fold, whereas in WT – 4.2-fold. Stomatal conductance after the action of HIL increased in WT and *hy4* in RL:BL option, while on BL the $g_s$ value practically did not change in both WT and the mutant (Table 1).

HIL irradiation reduced $PI_{ABS}$ values to the greatest extent in the *hy4* mutant of the BL option, and the reduction in WT was about the same compared with the other spectral variants. The difference between WT and *hy4* in the reduction of $PI_{ABS}$ values was approximately the same for the RL:BL option (1.9- and 2.6-fold, respectively), whereas in the GL-added variant, $PI_{ABS}$ values were reduced 1.9-fold in WT and 3.2-fold in the mutant. At the same time, in the variant with BL, $PI_{ABS}$ values in the mutant decreased 4-fold, and in WT 1.6-fold, that is, the decrease of the values in WT was markedly lesser than in *hy4*.

The $DI_0/RC$ parameter (Fig. 1*B*), which characterizes the dissipation of absorbed light energy mainly into heat, increased in all variants under the action of HIL and the increase was especially noticeable in *hy4* under all irradiation options. The most significant decrease of $F_v/F_m$ value was observed in the same options.

**Pro-/antioxidant balance**: The TBARS content of the mutant was higher compared to WT in all spectral options, except for RL:BL (Fig. 3*A*). Moreover, the TBARS content of WT was the lowest at the RL:BL:GL and that of *hy4* was the highest under BL compared with RL:BL:GL and RL:BL. Evaluation of the activity of one of the key antioxidant enzymes APX (Fig. 3*B*) showed this activity was lower in the mutant than in WT in all variants. Moreover, in WT, the activity of APX was the lowest at RL:BL:GL and the highest at BL. A difference in the activity of another key antioxidant enzyme GPX (Fig. 3*C*) between WT and *hy4* was found only in the RL:BL variant [0.45 ± 0.03 μmol $g^{-1}$(FM) $min^{-1}$ in WT and 0.28 ± 0.03 μmol $g^{-1}$(FM) $min^{-1}$ in *hy4*].

HIL irradiation resulted in an increase in TBARS content in WT and *hy4* in all spectral variants except WT in the RL:BL samples. The activity of APX increased significantly after irradiation in the RL:BL:GL mutant samples and under BL variants, whereas the changes were not significant in the *hy4* plants in the RL:BL option and WT in all options. Also, after 4 h of HIL treatment the activity of GPX increased in all variants in both WT and *hy4*, and the increase in activity was more significant in the WT compared with the mutant, especially a marked increase was observed in the options with the addition of GL and under BL.

The value of TEAC was the highest in the RL:BL variant, but the lowest in the *hy4* mutant under BL (Fig. 3*D*). The other spectral variants were intermediate between the two. HIL irradiation resulted in a 1.5-fold decrease in TEAC in the mutant in the RL:BL variant. In the other variants, the changes were not significant. However, under BL, both before and after HIL, TEAC values in WT were markedly higher than in *hy4*.

**Pigments**: The content of photosynthetic pigments before HIL irradiation was the same in the RL:BL samples and with the addition of GL both in WT and the mutant (Fig. 4). However, under BL, the content of these pigments was lower in the mutant than that in the WT. After irradiation of plants with HIL, the content of photosynthetic pigments did not change much. However, it also remained reduced in the *hy4* plants under BL.

Initially, the Chl *a*/*b* ratio was the highest in *hy4* at BL compared to other spectral variants (Table 2). Irradiation with HIL increased the Chl *a*/*b* ratio in WT and mutant in RL:BL samples but under BL this ratio did not change.

## Discussion

Photoinhibition is a light-induced decrease in photosynthetic activity of plants, algae, or cyanobacteria (Powles

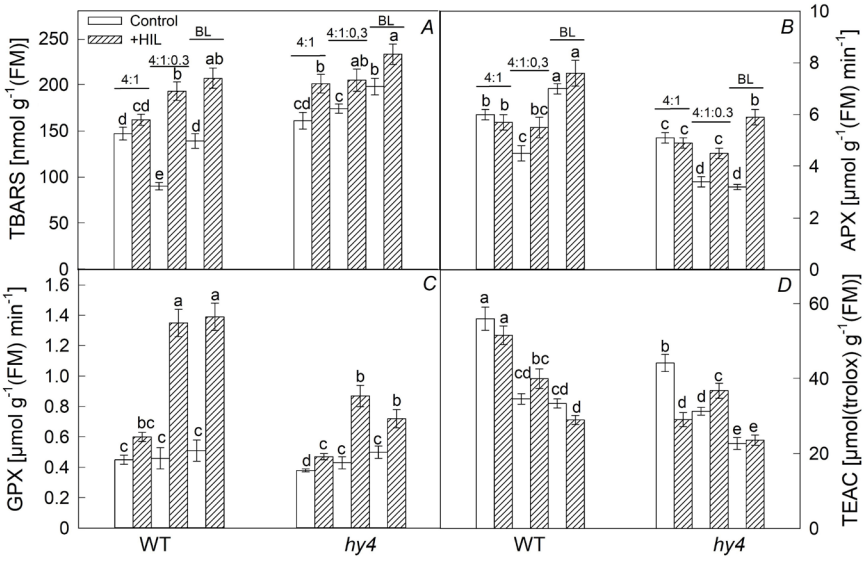


Fig. 3. Effect of high-intensity light (HIL) on the content of thiobarbituric acid reactive substances (TBARS) (*A*), activities of the antioxidant enzymes: ascorbate peroxidase (APX) (*B*) and guaiacol-dependent peroxidase (GPX) (*C*), and on Trolox-equivalent antioxidant capacity (TEAC) (*D*) in WT plants and the *hy4* mutant. Mean values ± SD are shown. *Different letters* correspond to significant differences in values at $p<0.05$, $n = 4$.

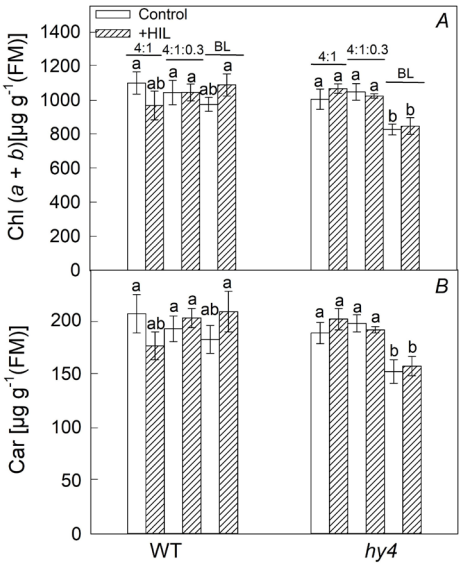


Fig. 4. Effect of high-intensity light (HIL) on Chl (*a*+*b*) (*A*) and carotenoid (*B*) contents in WT and *hy4* plants. Mean values ± SD are shown. *Different letters* correspond to significant differences in values at $p<0.05$, $n = 6$.

1984). PSII is more sensitive to strong light than other photosynthesis-related systems, so most researchers define the term 'photoinhibition' as light-induced damage to PSII (Liu *et al.* 2019). In photosynthetic organisms damaged by excessive light, PSII is continuously repaired by degradation and synthesis of the D1 protein of the photosynthetic reaction center of PSII (Nishiyama *et al.* 2006, Liu *et al.* 2020).

Photoreceptors, in particular cryptochromes, are known to play an important role in plant responses to stress factors (Carvalho *et al.* 2011, Liu *et al.* 2019). In particular, cryptochrome 1 has been suggested to play a key role in the response of PA to the action of HIL (Kleine *et al.* 2007, Kreslavski *et al.* 2009, 2020). However, the pathways of cryptochrome action in defense mechanisms are poorly understood. Investigation of the effect of cryptochrome 1 on PA status showed that this photoreceptor is important for the maintenance of PA resistance when plants are exposed to HIL-induced photoinhibition (Kleine *et al.* 2007, Kreslavski *et al.* 2020). Thus, Kleine *et al.* (2007) found that the PSII of *Arabidopsis* plants deficient in cryptochrome 1 was damaged already at 3 h of HIL irradiation [1,000 μmol(photon) $m^{-2}$ $s^{-1}$]. The PA of tomato plants was also found to be more sensitive to the

Table 2. Effect of high-intensity light (HIL) on chlorophyll (Chl) *a* and Chl *b* contents and their ratios in WT and *hy4* plants grown under LEDs of two spectral compositions: RL:BL = 4:1 and BL at a light intensity of 100 μmol(photon) $m^{-2}$ $s^{-1}$. Mean values ± SD are shown. *Different letters* correspond to significant differences in values at $p<0.05$, $n = 6$.

| Options/parameters | | Chl *a* [μg $g^{-1}$(FM)] | Chl *b* [μg $g^{-1}$(FM)] | Chl *a*/*b* |
|---|---|---|---|---|
| RL:BL = 4:1 | WT | 710 ± 37$^{a}$ | 389 ± 32$^{a}$ | 1.82 ± 0.11$^{b}$ |
| | WT+HIL | 653 ± 44$^{ab}$ | 314 ± 23$^{a}$ | 2.08 ± 0.12$^{a}$ |
| | *hy4* | 644 ± 31$^{ab}$ | 359 ± 32$^{a}$ | 1.79 ± 0.11$^{b}$ |
| | *hy4*+HIL | 723 ± 20$^{a}$ | 343 ± 10$^{a}$ | 2.03 ± 0.07$^{a}$ |
| BL | WT | 610 ± 17$^{ab}$ | 364 ± 22$^{a}$ | 1.68 ± 0.08$^{b}$ |
| | WT+HIL | 701 ± 32$^{a}$ | 388 ± 27$^{a}$ | 1.81 ± 0.10$^{b}$ |
| | *hy4* | 561 ± 21$^{b}$ | 265 ± 11$^{b}$ | 2.12 ± 0.09$^{a}$ |
| | *hy4*+HIL | 571 ± 29$^{b}$ | 275 ± 18$^{b}$ | 2.08 ± 0.10$^{a}$ |

negative effect of short-term HIL under cryptochrome 1 deficiency (Kreslavski *et al.* 2020). It is concluded that cryptochrome 1 plays an important role in protecting PA from HIL during long-term plant growth. It is unclear, however, to what extent the role of cryptochrome 1 in PA protection from HIL depends on spectral and other growing conditions, primarily the spectral light composition. Our data show that cryptochrome 1 deficiency does not significantly affect photosynthetic activity when RL:BL ratio = 4:1 (Fig. 1). However, when plants were additionally grown under BL, cryptochrome 1 deficiency significantly affected the photosynthetic rate ($P_N$), which was much higher in WT compared with *hy4*. Also, photosynthetic activity was higher in the variants RL:BL:GL = 4:1:0.3 in WT compared with the mutant. This fact is consistent with the higher TBARS content in the mutant in the RL:BL:GL and BL options compared to WT and the lower activities of the APX and GPX enzyme of *hy4* under these spectral conditions. The lowered value of $P_N$ in WT and mutant under HIL is not linked to stomatal conductivity as we can see from Table 1 since it was elevated or slightly changed under HIL. Also, there were no significant differences in stomatal conductivity between the *hy4* mutant and WT at RL:BL ratio = 4:1. Likely, cryptochrome 1 in opposite to phototropins (Mao *et al.* 2005) has a weak influence on stomatal conductance under our conditions and lowering $P_N$ at HIL is associated with nonstomatal effects.

The higher TBARS content in the mutant is probably due to higher photoinhibition of PSII, which is consistent with the lower values of the PSII performance index in *hy4* compared with WT. Similarly reduced activity of these enzymes and markedly increased PSII activity in WT compared with *hy4* were also found when the *Arabidopsis hy4* mutant was grown for a prolonged period under BL at 130 μmol(photon) $m^{-2}$ $s^{-1}$ (Kreslavski *et al.* 2021), which is close to the light intensity in our conditions. Another BL intensity used in the cited work was about 30 μmol(photon) $m^{-2}$ $s^{-1}$, which is slightly higher than under our conditions [20 μmol(photon) $m^{-2}$ $s^{-1}$ BL and 80 μmol(photon) $m^{-2}$ $s^{-1}$ RL]. At the same time, the difference in the activity of PSII in the mutant and WT decreased under the action of HIL, but this decrease in WT and the mutant was almost the same. That is this effect of lower PA resistance in *hy4*, as already mentioned, was not manifested at low intensity of BL (Kreslavski *et al.* 2021). The same trends were also revealed in our experiments but at a shorter duration of light exposure (3 d). This is consistent with the idea that cryptochrome 1 plays a key role in resistance to HIL at sufficiently high intensity of BL.

Walters *et al.* (1999) suggested that photoreceptors do not play an important role at the level of direct action on chloroplasts. However, it is clear from our data that cryptochrome 1 appears to regulate the content of photosynthetic pigments under BL-dominated conditions, as shown by the decrease in their content at BL (Fig. 4). In addition, under BL conditions cryptochrome 1 deficiency may affect the Chl *a*/*b* ratio (Table 2).

It is known that Chl *b* is found only in the antenna complex of PSII, so an increased relative content of Chl *b* leading to a decreased Chl *a*/*b* ratio ensures efficient leaf light harvesting in antenna complexes (Walters *et al.* 1999). Light harvesting decreases under HIL, as shown by the increased ratio, but this mechanism operates at RL:BL = 4:1 in both WT and the mutant, but not in *hy4* under BL. Moreover, light harvesting is impaired in *hy4* under BL compared with WT, whereas at RL:BL = 4:1, there is no difference in light-harvesting efficiency between WT and the mutant. This means that light harvesting by PSII LHC appears to be impaired under cryptochrome 1 deficiency.

It follows from our data that cryptochrome 1 deficiency leads to an increased sensitivity of PA to HIL at a high intensity of BL in the spectrum of light incidents on plants. This may be partly due to the lowered activity of major antioxidant enzymes such as APX and GPX and probably low-molecular-mass antioxidants (Kreslavski *et al.* 2021). Thus, when investigating the effect of cryptochrome 1 deficiency on the resistance of the PA mutant of *Arabidopsis hy4* to HIL, a reduced activity of APX and GPX was found in the mutant compared with WT when plants were grown for a long time under BL at 130 μmol(photon) $m^{-2}$ $s^{-1}$ (Kreslavski *et al.* 2021). However, this effect was not evident at low BL intensity, as mentioned above.

Plants demonstrate diverse photoprotective and adaptive mechanisms to avoid damage of PA, first of all, PSII caused by HIL or fluctuating light (Liu *et al.* 2019). The decrease in photosynthetic activity serves as one of the defense mechanisms against the negative effect of HIL on the PA, and this mechanism is more pronounced in the mutant. This fact is also consistent with the higher value of dissipation of absorbed light energy into heat ($DI_0$/RC), which was higher in *hy4* in the BL variant and with the addition of GL. This mechanism of dissipation also serves as one of the defense mechanisms (Ruban *et al.* 2016).

In summary, cryptochrome 1 deficiency and/or the presence of GL may lead to reduced activity of antioxidant enzymes such as APX and GPX, as well as the content of low-molecular-mass antioxidants, particularly carotenoids. This leads, under cryptochrome 1 deficiency or under conditions of additional GL, to a reduced resistance of the *Arabidopsis* PA to HIL.

The sensitivity of the PA to HIL is related to the content of the active form of cryptochrome 1, as well as to the presence or absence of shady cultivation conditions, which, according to several authors, occur with the addition of GL (Folta and Maruhnich 2007, Sellaro *et al.* 2010). It can be assumed that the addition of GL leads to a decrease in the content of the active form of cryptochrome 1 in both WT and the mutant. However, when cryptochrome 1 is deficient, it leads to a more marked decrease in low-molecular-mass antioxidants in the mutant than in WT. Therefore, the PA sensitivity of *hy4* to HIL is higher. Also, the higher sensitivity of the mutant PA to HIL may be because the spectral conditions become closer to shadow conditions upon the addition of GL (Wang and Folta 2013), resulting in a greater decrease in antioxidant potential in the mutant and a higher sensitivity of its PA to HIL.

As a result, cryptochrome 1 deficiency and/or the presence of GL may lead to reduced activity of antioxidant

enzymes such as APX and GPX, as well as the content of low-molecular-mass antioxidants, in particular, different pigments. The majority of leaf pigments being cell antioxidants or optical absorbing excess energy filters play an important role in protection mechanisms from HIL (Havaux and Kloppstech 2001). This agrees with data that low carotenoid contents may decrease PA resistance owing to a diminished capacity to absorb excess excitation energy or neutralize triplet Chl, leading to decreased oxidative stress (Ruban *et al.* 2016, Simkin *et al.* 2022). Resulting in reduced content of carotenoids and value of TEAC, also diminished antioxidant enzyme activity leads to a shift of pro-/antioxidant balance towards oxidants under cryptochrome 1 deficiency or under the presence of additional GL. As a result, the resistance of *Arabidopsis* PA to HIL decreases.

**Conclusion**: Thus, even relatively short-term cultivation of *Arabidopsis* plants at moderate BL leads to a stronger photoinhibition in the cryptochrome 1-deficient mutant compared with WT and other spectral conditions, whereas in WT, the photoinhibition of PSII activity was approximately the same regardless of the spectral conditions (different ratios of RL, BL, and GL). This means that cryptochrome 1 is of key importance in the presence of sufficiently high intensity of BL in the emission spectrum, whereas at low intensity of BL the degree of photoinhibition in WT and the mutant is not much different. We think that there is a link between lower antioxidant activity and cryptochrome and phytochrome-mediated signaling, which in the case of cryptochrome 1 shows up well at high BL. This link is realized primarily through the photoreceptor-induced expression of genes encoding antioxidant enzymes and pigment-biosynthesis enzymes (Kleine *et al.* 2007, Kreslavski *et al.* 2018). This is probably because the total activity of key antioxidant enzymes and the content of low-molecular-mass antioxidants in WT and the cryptochrome 1-deficient mutant differ the most strongly at high enough BL.

From the obtained and previously published data (Kreslavski *et al.* 2021), we can conclude that when growing *Arabidopsis* plants under sufficiently strong light, it is necessary to use radiation with a high proportion of BL.

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