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Impact of additional green light and deficit in cryptochrome 1 on photosynthetic activity and pro-/antioxidant balance in *Arabidopsis thaliana*

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Abstract

The light spectral composition acting through a set of photoreceptors, such as cryptochromes and phytochromes, plays an important role in maintaining sustainable photosynthesis. An impact of cryptochrome 1 deficiency and additions of green light (GL) against the background of red (RL) and blue (BL) (different ratios of RL:BL:GL) on the activity of the photosynthetic apparatus, the content of photosynthetic pigments, pro-/antioxidant balance, and expression of some genes in the leaves of 23-d-old *Arabidopsis thaliana hy4* mutant plants was studied. The deficiency of cryptochrome 1 at RL/BL ratio of 4:1 led to a decrease in the rate of photosynthesis, photosystem II activity, and activity of ascorbate peroxidase and total peroxidase but to an increase in the content of products reacting with thiobarbituric acid. However, in the presence of additional GL, this difference for photosynthetic parameters either decreased or was absent, likely due to a GL-induced decrease in the content of active cryptochrome.

Keywords: cryptochrome mutant; photosynthesis; photosystem II.

Introduction

The spectral composition of light plays an important role in the regulation of many physiological processes in plants, primarily for maintaining photosynthetic processes at a sufficiently high level and for the accumulation of biologically active compounds. The effect of light of different spectral composition on various metabolic

Highlights

- Additional green light reduced photosynthetic activity in *Arabidopsis* wild type
- The addition of green light did not affect photosynthetic activity in the *hy4* mutant
- Cryptochrome 1 deficit reduced photosynthetic activity in *Arabidopsis*

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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; HIL – high-intensity light; PA – photosynthetic apparatus; PI_{ABS} – PSII performance index; POD – peroxidase; RL – red light; TBARS – thiobarbituric acid reactive substances; TEAC – trolox equivalent antioxidant capacity; UVAPs – ultraviolet-absorbing pigments.

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processes in plants is realized using a known set of cellular photoreceptors, such as red/far-red light receptors – phytochromes and blue-UV-A receptors – cryptochromes and phototropins. However, no specific green light (GL) photoreceptor was found in many studies (Li *et al.* 2021). On the other hand, direct action of red light (RL), blue light (BL), or GL is also possible.

Three genes of cryptochromes encoding photosensitive proteins Cry1, Cry2, and Cry3 were found in the genome of *Arabidopsis thaliana* plants (Liu *et al.* 2011). Cryptochromes 1 and 2 are key ones and regulate the plant growth and photomorphogenesis as well as biosynthesis of many photosynthetic proteins and enzymes, especially the key enzyme of the Calvin cycle, Rubisco (Lin and Todo 2005, Chaves *et al.* 2011, Liu *et al.* 2011, 2016; D'Amico-Damião and Carvalho 2018, Voitsekhovskaja 2019).

It is known that *hy4* mutants with a deficit of cryptochrome 1 exhibit decreased sensitivity to BL, whereas transgenic plants overexpressing it show increased photosensitivity (Lin *et al.* 1998). Wherein, cryptochrome 2 functions mainly under low intensities of BL in the early photomorphogenesis of *Arabidopsis* seedlings. Under our conditions [100 μmol(photon) m^{-2} s^{-1}], the degradation of cryptochrome 2 in the wild type (WT) could be possible whereas the content of cryptochrome 1 in WT would be constant. Therefore, the *hy4* mutant is used in some studies.

Together with the COP1 (multifunctional ubiquitin ligase, CONSTITUTIVELY PHOTOMORPHOGENIC 1) and HY5 transcription factors, cryptochrome 1 is involved in the induction of plant responses to the high-intensity BL (Kleine *et al.* 2007). Conversely, cryptochrome 2 photoreceptor functions at low BL intensities. Also, cryptochrome 1 in some physiological processes is an indicator of the BL/GL ratio in the incident light spectrum (Sellaro *et al.* 2010, Wang and Folta 2013). Analysis of gene expression in the response to high-intensity light (HIL) revealed the regulatory role of cryptochrome 1 in the response of many genes to HIL (Kleine *et al.* 2007). Cryptochrome 2, on the contrary, degrades even in low light and is involved in photoperiodism (D'Amico-Damião and Carvalho 2018, Fantini *et al.* 2019). Also, it is known that *hy4* mutants with a deficit of cryptochrome 1 exhibit decreased sensitivity to BL, whereas transgenic plants overexpressing *Cry1* show increased photosensitivity (Lin *et al.* 1998). Wherein, cryptochrome 2 functions mainly under low intensities of blue light in the early photomorphogenesis of *Arabidopsis* seedlings. Therefore, the *hy4* mutant was used in some studies at moderate and strong light (Kleine *et al*. 2007, Kreslavski *et al*. 2021).

Cryptochromes form part of the plant photoregulatory system that controls a wide range of different processes, including those associated with plant adaptation and their photosynthetic apparatus (PA), in particular, some plant stress responses, such as drought, salinity, elevated temperatures, and HIL (Kleine *et al.* 2007, D'Amico-Damião and Carvalho 2018), biosynthesis of various pigments, including anthocyanins and chlorophyll (Chl) (Yang *et al.* 2000, Giliberto *et al.* 2005).

It is important to note that cryptochrome is a sensor of BL and the ratio of BL to GL, and this was demonstrated in the example of hypocotyl elongation in *Arabidopsis* plants (Sellaro *et al.* 2010). Also, a relationship was found between changes in the ratio of BL to GL and changes in the content of anthocyanins (Zhang and Folta 2012) and phenolic compounds (D'Amico-Damião and Carvalho 2018) in the leaves of *Arabidopsis* and lettuce plants.

It is known that GL sensors regulate development and growth with red and blue photosensors (Folta and Maruhnich 2007). However, less information is obtained concerning photosynthesis and antioxidant status under different BL/GL ratios in the emission spectrum, which influences, as we assumed, the activity of cryptochrome 1 in the regulation of photosynthetic processes and pro-/antioxidant balance. The last is important for adaptation under different light spectral conditions and photosynthesis, maintaining plant growth and development by providing energy and various substances. However, the role of the photoreceptors in the light regulation of these processes under both stressful and physiological conditions has not yet been sufficiently studied.

Note that both cryptochromes and phytochromes absorb in the green part of the spectrum. However, the absorbance of phytochromes in the green spectral region is too small compared to those in the red part of the spectrum (Kreslavski *et al.* 2009).

It was demonstrated in the previous work (Kreslavski *et al.* 2021) that the PA of *A. thaliana hy4* mutant grown under moderate BL was more sensitive to high irradiance and UV-B than the WT. Wherein, the content of carotenoids and ultraviolet-absorbing pigments (UVAPs) and peroxidase activity in the *hy4* mutant were smaller but the content of thiobarbituric acid-reactive substances (TBARS) was much higher than that in the WT. This is one of the reasons for the increased resistance of the *A. thaliana* WT. This is consistent with the study of Kleine *et al.* (2007), who suggested a novel function of cryptochrome 1 in mediating plant responses to high irradiances that are essential to the induction of photoprotective mechanisms.

It would be important to understand how the content of the active form of cryptochrome 1, which depends on the ratio of BL to GL, affects photosynthetic processes and pro-/antioxidant balance at different BL/GL ratios. In the present work, the role of cryptochrome 1 and BL/GL ratio in photosynthetic processes and pro-/antioxidant balance in the *hy4* mutant *A. thaliana* was studied. Also, the role of some light-induced genes in these processes was examined.

Materials and methods

Cultivation of plants and scheme of the experiment: The experiments were conducted with *Arabidopsis thaliana* (Col-0 ecotype) WT and *hy4* mutant deficient in cryptochrome 1 (catalog no. CS70). Plant seeds were obtained from *Nottingham Arabidopsis Stock Center* (Nottingham, UK). First, all plants were grown for 20 d under LEDs with a ratio of RL:BL = 4:1. Then one part

of the plants was transferred to light for 3 d with a ratio of RL:BL:GL = 4:1:0.3, the second part was exposed to the light of RL:BL:GL = 4:1:1, the third part to the ratio of RL:BL:GL = 3:1:2, and part of the plants remained in the original light (RL:BL = 4:1) (Fig. 1). In all experiments, the plants were grown with a 12-h photoperiod at 23 ± 1°C during the day and 21 ± 1°C at night at a similar light intensity of 100 μmol(photon) m^{-2} s^{-1}.

Photochemical activity: Fluorescence parameters were assessed by the JIP test using the fluorometer described in Kreslavski *et al.* (2014). The values of F_0, F_v, and F_m were determined, where F_m and F_0 are the maximum and minimum levels of Chl fluorescence under dark-adaptation conditions, respectively, F_v is the photoinduced change in fluorescence. Based on the obtained OJIP induction curves the values of F_v/F_m, DI_0/RC, and PI_{ABS} were determined (Stirbet and Govindjee 2011, Goltsev *et al.* 2016). Here, F_v/F_m is the maximum photochemical quantum yield of PSII, DI_0/RC = (ABS/RC) – (TR_0/RC), total energy dissipated by a PSII reaction center, and PI_{ABS} is the performance index, which is equal to (ABS/RC) × (F_v/F_0) × [$ET_0/(TR_0 - ET_0)$], reflecting the photochemical activity of PSII. TR_0/RC is the maximum (initial) exciton flux captured by all reaction centers of PSII per photochemically active PSII reaction center, ET_0 is electron flux from Q_A to Q_B, ABS/RC is the average absorbed light flux by PSII antenna Chl per photochemically active reaction center of PSII.

CO_2 gas exchange: The rate of photosynthesis was determined using a portable *LCPro+* gas-exchange system (*ADC BioScientific Ltd.*, UK) in an open system at a temperature of 21.5 ± 0.5°C, the CO_2 concentration of 420 ± 12 μmol m^{-2} s^{-1}, and relative humidity of 75–80%. The measurements were carried out at a light intensity of 600 μmol(photon) m^{-2} s^{-1}. All measurements were performed from 9:00 to 13:00 h. The photosynthetic rates were recorded for 6–8 min until stable values were reached.

Content of photosynthetic and UV-absorbing pigments: The content of Chl *a*, *b*, and carotenoids was determined according to the method of Lichtenthaler (1987). The absorption of the samples was measured on a *Genesys 10 UV* spectrophotometer (*Thermo Fisher Scientific*, USA) at λ_M – 470, 649, and 665 nm.

The content of UVAPs was determined in fully developed leaves (8–12), which were kept for 24 h in acidic methanol (methanol:water:HCl, 78:20:2) at +4°C (Mirecki and Teramura 1984). The optical density of the samples was determined in the maximum UV range (about 327 nm) using a spectrophotometer (*Genesys 10 UV*, *Thermo Fisher Scientific*, USA). The content of UVAPs was expressed in relative units per 100 mg of fresh mass (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) by reducing the absorption at 290 nm as a result of the oxidation of ascorbate. The activity of guaiacol-dependent peroxidase (POD, EC 1.11.1.9) was measured as described in Balakhnina and Nadezhkina (2017) based on the conversion of guaiacol to its oxidized tetra guaiacol form and monitored at 470 nm. The content of TBARS was determined according to the method described in Balakhnina and Nadezhkina (2017). The absorption of TBARS was measured at 532 and 600 nm using a *Hitachi-557* spectrophotometer (Kyoto, Japan). All results are based on 1 g of FM.

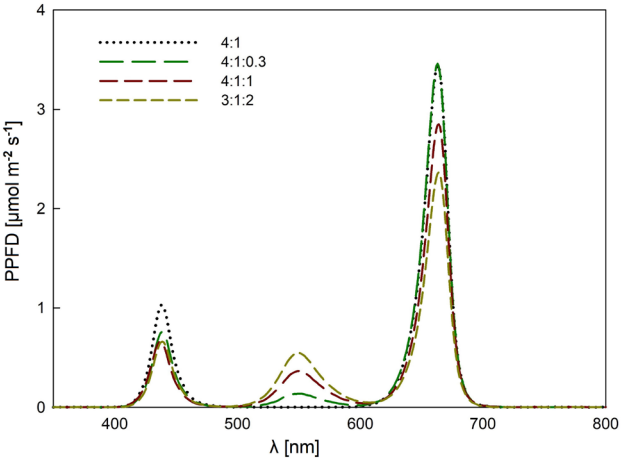

Fig. 1. Emission spectra of LEDs used for plant growth.

Trolox equivalent antioxidant capacity (TEAC): TEAC was determined spectrophotometrically according to the method described in Re *et al.* (1999) involving the reaction of methanolic extracts with 2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt (ABTS) (*Sigma-Aldrich*, Burlington, MA, USA, CAS no. 30931-67-0). The antioxidant capacity of low-molecular-mass antioxidants was expressed in μmol(trolox) g^{-1}(FM).

Quantitative real-time PCR: RNA isolation was performed according to the method of Kolosova *et al.* (2004) and Pashkovskiy *et al.* (2019). The quantity and quality of the total RNA were determined using a *NanoDrop 2000* spectrophotometer (*Thermo Fisher Scientific*, USA). cDNA synthesis was performed using the *M-MLV Reverse Transcriptase Kit* (*Fermentas*, Canada) and the oligo (dT) 21 primer for nuclear coding genes and random 6 for chloroplast genes. The expression patterns of the genes were assessed using the *CFX96 Touch™ Real-Time PCR Detection System* (*Bio-Rad*, USA). The transcript levels were normalized to the expression of the *Actin1* gene. The relative gene expression signal intensity in WT plants (RL:BL = 4:1) was taken as 1. Primer sequences are presented in the text table.

Statistics: For fluorescent and photosynthetic measurements, fully developed, healthy-looking upper leaves with nearly horizontal leaf blades were used. In each variant,

	NCBI RefSeq	Gene	Forward	Reverse
1	NM_001123772.2	*APX1*	CTCATGGAGCCAACAGTGGT	GGAAAGGAATGTCAGGGCCA
2	NM_119666.4	*APX3*	TCTCGATCTCTGTGAGGGCG	TTCTCCCGGGAACGAACA
3	NM_001342391.1	*APX5*	ACCGTCCACACAACAAAGGT	CGGCCTGGTGTAAATGGGAT
4	NM_121396.4	*CHS*	CCAAGCTTCTTGGTCTCCGT	ACGTGCTCCACGATTGTTCT
5	NM_128855.4	*COP1*	CCCTGTTGTAGCCAACACCT	TCACAACCCCTTTGTAGTGCT
6	NM_001084700.2	*HYH*	ACTGGTAGGTGTTGAAAGAAACTT	AGCTCCGGATTGTTGACTCC
7	AB005456.1	*HY5*	GCTTAGACATATTCTGAAGAACACA	GATCAAAGGCTTGCATCAGCA
8	NM_102733.3	*CAB1*	AGACATTCGCAAGGAACCGT	AATGCTCTGAGCGTGAACCA
9	NM_001331837.1	*PIF3*	GCCCATCCGAAAGTCCTTCA	CCAAACCCGTTCGAGATGGA
10	NM_129862.3	*PIF4*	TGCATCACAACCGACCGTAA	AACTTCAGCTGCTCGACTCC
11	NM_124799.4	*PORa*	ATGCTTCAGCATCATCATCATTCAA	TCAAGCTCTGTTCCCTCTTGC
12	NM_118879.4	*PORb*	GCGACTTCAAGCCCTACAGT	GCACGCCATTATCACGTTCC
13	NM_100243.4	*PORc*	AGAGAAGACAGAAACCGCGA	AACCAGACGAAGCTCCAGTG
14	NP_051039.1	*psbA*	GAGGAGCAGCAATGAATGCG	GCGAAAGCGAAAGCCTATGG
15	NP_051054.1	*psbD*	CTTTTGTTGCTCTCCACGGC	GCGCAAAGAACCAACCAGAT
16	NM_100828.4	*phyA*	GGTTAGCCGGAAACTGGTGA	TTGTTTGCTGCAGCGAGTTC
17	NM_127435.4	*phyB*	GGATTCAACAGCTCCTGGCT	ACGCCATTCTGAATTCTGTGC
18	NM_122975.3	*phyC*	TGAGGGAAAGGTTACCGGGG	TTGCCTTTTCGGGGTCCTTC
19	NM_117721.2	*phyD*	GCGATTCTCCACAGGGTTGA	TTAATGTCGCCGCTAGGCAA
20	NM_117923.8	*phyE*	ACTAAGGGTTGAAGTCGCCG	CCTGCCCTGGTGAGATACTG
21	NM_001036427.3	*Act1*	TTAGCAACTGGGATGACATGGA	CCTGAATGGCAACATACATAGCA

6–12 healthy developed upper leaves from 3–4 plants were used. At least 10–15 leaves were used for each treatment to measure pigment content and mass. All experiments were repeated three or four times (*n*). Data in tables and figures are expressed as mean ± SD. One-way analysis of variance (*ANOVA*) by *Duncan*'s method was performed using the *SigmaPlot 12.3* program (*Systat Software Inc.*, USA). Different letters were used to denote significant differences between WT and mutants at $p<0.05$. The differences between the two variants were analyzed by the *Student*'s *t*-test at the 5% significance level.

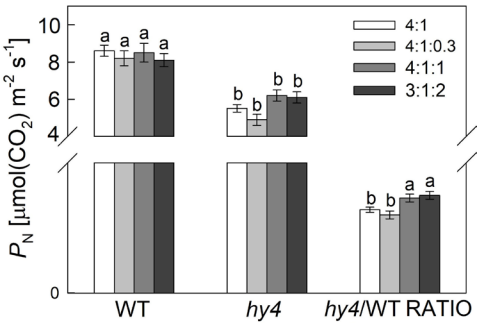

Fig. 2. Photosynthetic rate (P_N) of 23-d-old wild type *Arabidopsis thaliana* (WT) and *hy4* mutant plants grown under LEDs with ratios of RL:BL = 4:1, RL:BL:GL = 4:1:0.3, RL:BL:GL = 4:1:1, and RL:BL:GL = 3:1:2 at a light intensity of 100 μmol(photon) m^{-2} s^{-1}, photoperiod of 12 h. Mean values ± SD are shown. *Different letters* correspond to a significant difference in values at $p<0.05$, $n = 4$.

Results

Photosynthetic activity: Comparison of photosynthetic rates (P_N) showed that there is no noticeable difference between P_N values in 23-d-old *Arabidopsis* WT plants grown under different spectral conditions in different ratios of RL, BL, and GL (Fig. 2). A similar trend was also found for the *hy4* mutant. However, in all variants, the P_N values in the mutant were lower than the corresponding values in the WT. The ratios of the rates of photosynthesis in the *hy4* mutant to the corresponding rates in the WT among the variants with additional GL were the lowest at the RL:BL:GL ratio = 4:1:0.3. Another pattern was observed for the values of the PSII performance index PI_{ABS} in WT and *hy4*, *i.e.*, there was no noticeable difference between these indexes in WT or mutant plants grown in light of different spectral composition with added GL (Fig. 2*C*). Thus, in contrast to the variant with P_N, there was no significant difference in PI_{ABS} values between WT and the *hy4* mutant. Similar trends as for PI_{ABS} were found for F_v/F_m values (Fig. 3*B*). The PSII maximum quantum yield was the highest in WT at a ratio of RL:BL = 4:1 and it was greater than the F_v/F_m values of the mutant in all variants at this ratio (Fig. 3*A*). All other WT options were mainly intermediate between the above options (Fig. 3). However, we did not indicate a significant difference between the value of DI_0/RC in WT and *hy4*, excluding RL:BL ratio 4:1, where this parameter for WT was higher than that for the *hy4* mutant.

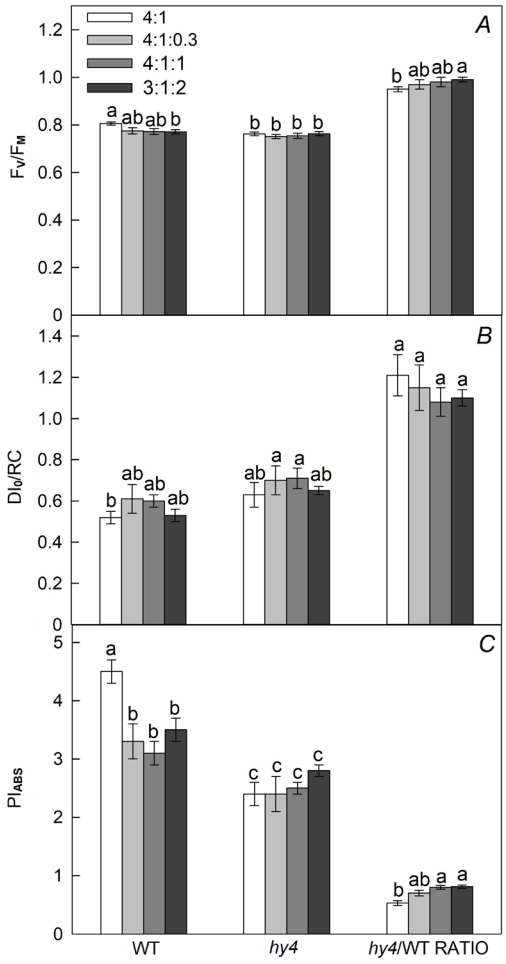

Fig. 3. Fluorescence parameters: F_v/F_m (*A*), DI_0/RC (*B*), PI_{ABS} (*C*) in WT and *hy4* mutant plants. Mean values ± SD are shown. *Different letters* correspond to a significant difference in values at $p<0.05$, $n = 4$. Here, F_v/F_m – PSII maximal quantum yield, DI_0/RC – quantum yield of energy dissipation, PI_{ABS} – PSII performance index.

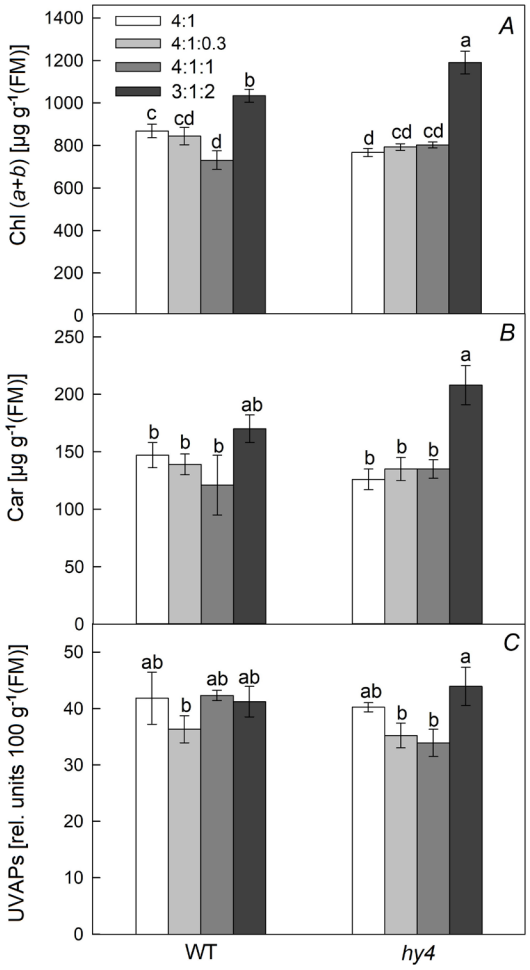

Fig. 4. Content of Chl (*a*+*b*) (*A*) and carotenoids (Car) (*B*) and UV-absorbing pigments (UVAPs) (*C*) in WT and *hy4* mutant plants. Mean values ± SD are shown. *Different letters* correspond to a significant difference in values at $p<0.05$, $n = 4$.

Pigments: The content of Chl (*a*+*b*) and carotenoids was maximal for plants grown on RL:BL:GL ratio = 3:1:2 (Fig. 4*A*,*B*). In all other spectral variants, the content of these pigments in WT and the mutant did not differ much, excluding the initial variant (RL:BL = 4:1). A significant difference in the content of UVAPs between WT and the mutant was manifested only at RL:BL:GL ratio = 4:1:1 (Fig. 4*C*).

Pro-/antioxidant balance: The value reflecting the activity of leaf low-molecular-mass antioxidants (TEAC) was maximum in the mutant at the RL:BL:GL ratio = 4:1:0.3, *i.e.*, 6.4 ± 0.3 µmol(trolox) g^{-1}(FM) and for WT at RL:BL ratio 4:1, it was 6.8 ± 0.3 µmol(trolox) g^{-1}(FM) (Fig. 5). A significant difference between WT and *hy4* was observed only at a ratio of RL:BL = 4:1, while the options of RL:BL:GL = 4:1:1, RL:BL:GL = 3:1:2, and RL:BL:GL = 4:1:0.3 did not differ significantly from each other.

It is important to note that the ratios of P_N, PI_{ABS}, and F_v/F_m in the mutant to those in the WT, increased as the share of GL in the spectrum of the light source increased. The corresponding ratios of TEAC values also increased as the share of GL increased.

The content of TBARS in the mutant compared to WT was significantly higher in all spectral options (Fig. 6*A*). At the same time, no difference was found in the content of TBARS between different variants in WT, however, in the WT with a ratio of RL:BL:GL = 3:1:2, the content of TBARS was the lowest. Evaluation of the activity of one of the key antioxidant enzymes, APX showed that at any RL:BL:GL ratio, APX activity did not noticeably differ, except for two options: the activity in *hy4* at RL:BL:GL ratio = 3:1:2 was the lowest and the activity in WT at RL:BL = 4:1 was the highest (Fig. 6*B*). The activity of another key antioxidant enzyme, POD, was maximal in WT at RL:BL:GL ratio = 4:1:1 [1.53 ± 0.07 µmol g^{-1}(FM) min^{-1}], somewhat less at an RL:BL:GL ratio = 3:1:2 [1.28 µmol g^{-1}(FM) min^{-1}], while in other cases the activity

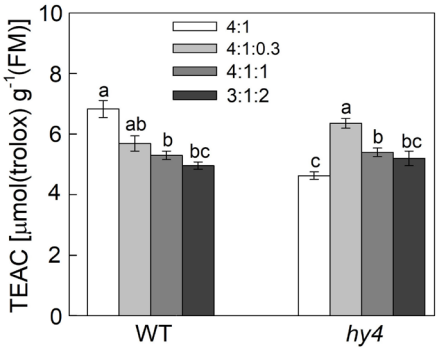

Fig. 5. Trolox-equivalent antioxidant capacity (TEAC) in WT and *hy4* mutant plants. Mean values ± SD are shown. *Different letters* correspond to a significant difference in values at $p<0.05$, $n = 4$.

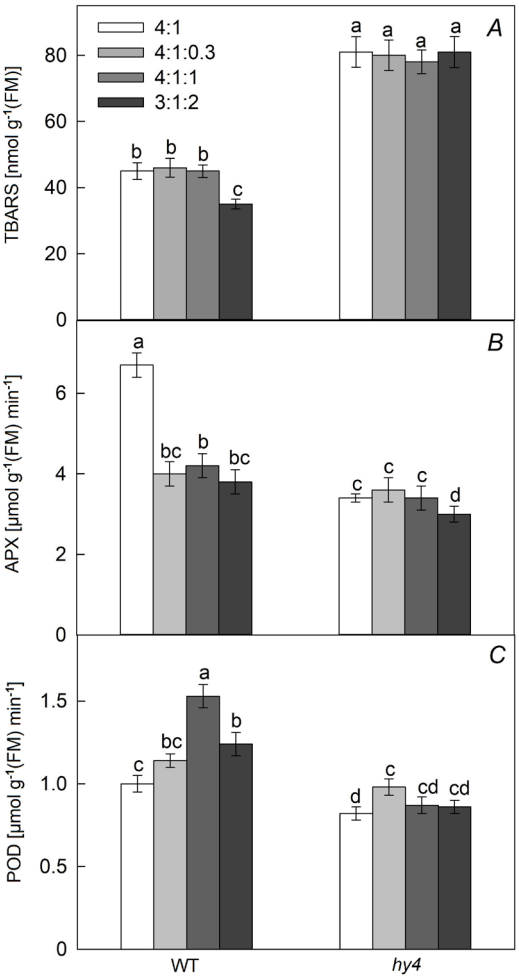

Fig. 6. The content of thiobarbituric acid reactive substances (TBARS) (*A*) and also activity of the antioxidant enzymes ascorbate peroxidase (APX) (*B*) and guaiacol-dependent peroxidase (POD) (*C*) in the leaves of WT and *hy4* mutant plants. Mean values ± SD are shown. *Different letters* correspond to a significant difference in values at $p<0.05$, $n = 4$.

was lower and fluctuated within the range [0.82–1.0 µmol g^{-1}(FM) min^{-1}] (Fig. 6*C*).

Expression of genes: It was shown that the levels of expression of the *APX1*, *APX3*, *APX5* genes were enhanced (an increase by 3–4 times) in WT at RL:BL:GL ratio = 4:1:0.3 (Fig. 7*A*). The expression of the *CHS* gene was reduced in the *hy4* mutant at RL:BL:GL ratio = 4:1:0.3 (Fig. 7*A*). The transcript levels of the *psbA* and *psbD* genes increased more than 2-fold in the mutant at RL:BL:GL ratio = 4:1:0.3 (Fig. 7*B*). The transcript level of the *CAB1* gene increased more than 3-fold in WT at RL:BL:GL ratio = 3:1:2 (Fig. 7*B*). It should be noted that in the variant at RL:BL:GL ratio = 3:1:2, transcript level of *PORc* gene increased in the WT, which was not observed in other variants (Fig. 7*C*). In the mutant, the expression level of the *PORa* gene was the highest at the RL:BL:GL ratio = 3:1:2. The expression levels of the *HY5*, *HYH*, *COP1*, *PIF3*, and *PIF4* genes in WT at RL:BL:GL ratio = 3:1:2 were higher (by about 2–3 times) than in the control (WT with RL:BL ratio = 4:1), while in all other options these levels were close to the control (Fig. 7*D*).

It is also important to note that in all spectral options, the levels of expression of the apoprotein genes of phytochromes D and C increased sharply concerning the control, while the levels of the genes encoding apoproteins of other phytochromes (phyA, phyB, phyE) did not increase (Fig. 7*E*).

Discussion

There is a lot of works studying the role of cryptochromes in maintaining sustainable photosynthesis and needed antioxidant potential during plant growth and development (Kleine *et al.* 2007, Kreslavski *et al.* 2020, 2021).

The phytochrome-mediated responses to far-red light affecting the phytochrome system have been well described (Demotes-Mainard *et al.* 2016, Cao *et al.* 2018, Shmarev *et al.* 2020). Less described are the GL responses and those that are blue-green reversible (Wang and Folta 2013), especially a relationship between the cryptochrome-dependent action of GL and changes in BL/GL ratio on photosynthetic processes and pro-/antioxidant balance.

The role of cryptochrome 1 deficiency at different RL:BL:GL ratios were studied using the most typical ratio of RL to BL equal to 4:1 (Zhang *et al.* 2019, Li *et al.* 2021). In this case, additional GL was about 6, 17, and 30% of the total incident light intensity. For example, in the study of Li *et al.* (2021), the effect of different lights on the morphology and photosynthetic traits of lettuce plants was studied at different shares of GL at fixed RL:BL ratio = 4:1.

Without the addition of GL, we observed lowered PSII photochemical activity, photosynthetic rate, and Chl (*a*+*b*) content in the mutant compared to the WT. This means that cryptochrome 1 is important to support photosynthetic activity. In addition, the content of TBARS was higher in the mutant than that in the WT, whereas the activity of antioxidant enzymes was lower. Together

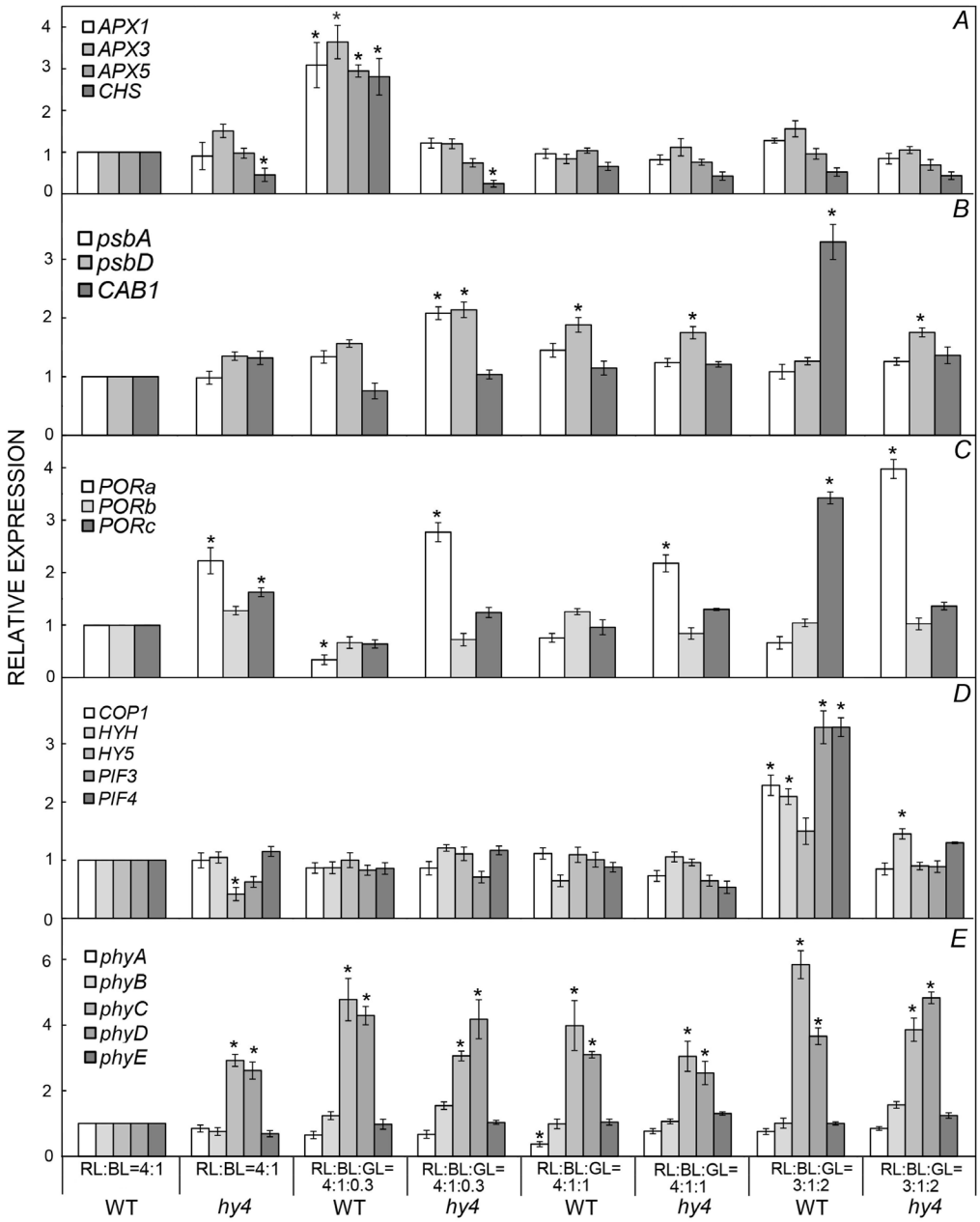

Fig. 7. Transcript levels of antioxidant enzymes (*APX1*, *APX3*, *APX5*, *CHS*) (*A*), photosynthetic proteins and enzymes (*psbA*, *psbD*, *CAB1*, *PORa*, *PORb*, *PORc*) (*B*,*C*), transcription factors involved in light signaling (*COP1*, *HYH*, *HY5*, *PIF3*, and *PIF4*) (*D*), and phytochromes (*phyA*, *phyB*, *phyC*, *phyD*, *phyE*) (*E*) in the leaves of WT and *hy4* mutant. The levels of WT at RL:BL = 4:1 were taken for 1. * – the difference between WT at RL:BL ratio = 4:1 and seven other spectral options is significant, $p<0.05$, $n = 3$.

with lowered maximal quantum PSII yield the data point out the development of weak stress in the mutant under these light conditions. We suggest that cryptochrome 1 is important for maintaining photosynthetic activity but due to the significant contribution of GL the content of active cryptochrome decreases and this activity is lowered (Figs. 2, 3). The same suggestion on the significant role of cryptochrome 1 for maintaining *Arabidopsis* PA resistance under HIL-induced stress was expressed in some works (Kleine *et al.* 2007, Kreslavski *et al.* 2020). Thus, sensitivity of PA to stress induced by HIL increased in cryptochrome 1-deficient *Arabidopsis* plants, provided that the plants were grown on BL at an intensity of 130 μmol(photon) m^{-2} s^{-1}, but not 30 μmol(photon) m^{-2} s^{-1}. In this work, the difference in the response of PA to stress was explained by the fact that when plants were grown on weak BL, no difference was found in the peroxidase and catalase activity of leaves and the content of UVAPs having antioxidant activity, while at a HIL, the content of UVAPs and the antioxidant enzymes activities were higher

than that in the cryptochrome 1-deficient mutant. In our case, activities of POD and APX, and value of the TEAC (at 4:1 ratio) in the *hy4* mutant were lower than those in the WT which can explain lowered PSII activity in the mutant. Another reason for the reduced photosynthetic activity in the *hy4* mutant can be the effect of GL on the WT stomatal opening. It is known that BL stimulates stomatal opening (Wang and Folta 2013), and GL can reverse stomatal opening responses induced by BL or decrease stomatal conductance in *Arabidopsis thaliana*. This effect of GL can be one of the reasons for the reduced difference in P_N between the WT and *hy4* mutant when adding GL to the spectrum.

Although GL is less efficient in the induction of photosynthesis due to the weak absorption of GL by photosynthetic pigments, there is now significant evidence of an important role for GL in plant biology (Wang and Folta 2013, Smith *et al.* 2017). For example, GL is a signal to slow down or stop some growth processes (Folta and Maruhnich 2007) affecting plant physiological processes *via* cryptochrome-dependent and cryptochrome-independent paths (Folta and Maruhnich 2007, Sellaro *et al.* 2010). Thus, illumination of plants with GL (maximum 563 nm) led to the inactivation of the cryptochome active form (Bouly *et al.* 2007) and it is important for cryptochrome-dependent mean. This is agreed with the data of Sellaro *et al.* (2010) who studied the impact of BL/GL ratio on cryptochrome-mediated inhibition of hypocotyl growth in *Arabidopsis*; they concluded that cryptochrome is a sensor of BL and BL/GL ratio. However, it is largely unclear how cryptochrome under different BL/GL ratios affects photosynthetic processes and pro-/antioxidant ratio.

It is known that GL might be useful since it can better penetrate plant canopy increasing plant growth and biomass accumulation by enhancing the contribution of photosynthesis from lower tiers (Kim *et al.* 2004). Therefore, we used the upper leaves to escape the effect of the plant canopy. A moderate proportion of GL can optimize the photosynthesis of plant leaves as a result of the enhancement of length and leaf size of plants and hence elevated quanta absorption, especially in lower tiers (Smith *et al.* 2017, Li *et al.* 2021).

However, due to GL absorbing in an open leaf not so well, we observed lowered photosynthetic activity in WT with a significant contribution of GL in falling light irradiation (RL:BL:GL = 3:1:2 ratio) but in the mutant, we did not indicate a significant difference between light conditions. This demonstrates the importance of cryptochrome 1 in the effects of GL (through likely BL/GL ratio) on photosynthetic processes.

TBARS content is one of the key biomarkers of oxidative stress (Şen 2012). Comparing the data obtained in the previous study of Kreslavski *et al.* (2021) with our results, we see that at any spectral combination, the TBARS content in the *hy4* mutant leaves was higher than those in the WT but the peroxidase activity was smaller. However, the value of TEAC was higher in the WT only in the absence of GL at RL/BL ratio equal to 4:1. Enhanced formation of TBARS reflects a grade of development of oxidative stress (Şen 2012). We can see a significant difference between the WT and *hy4* mutant, namely independently on light quality, a grade of oxidative stress in a *hy4* mutant leaf was almost twice as high as in the WT. However, in our opinion, in chloroplasts at a high fraction of GL in the spectrum, this difference is reduced. This suggestion is consistent with the reduced difference in photosynthetic activity and value of the TEAC between the WT and *hy4* mutant in the presence of GL. We suggest GL-reducing cryptochrome 1 activity can decrease the chloroplast photosynthetic activity. We found that the transcription of the ascorbate peroxidases of various localization *APX1*, *APX3*, and *APX5*, as well as the expression of a key enzyme in flavonoid biosynthesis chalcone synthase gene (*CHS*) was enhanced in the WT at a ratio of RL:BL:GL = 4:1:0.3 (Fig. 7*A*). Apparently, this increase is one of the mechanisms for maintaining photosynthetic activity in the WT. In addition, the value of TEAC was higher in WT at RL:BL ratio = 4:1 compared to other options. This agrees with the hypothesis on cryptochrome 1 controlling antioxidant activity.

An interesting fact is an increase in the expression of phytochromes C and D both under conditions of exposure to RL+BL and with additional GL; the more radiant the spectrum, the stronger the increase in expression. It can be assumed that phytochromes C and E, regardless of cryptochrome 1, are involved in green spectrum light signaling, which expands their regulatory potential.

We assume that the content of the active form of cryptochrome, regulated by the BL/GL ratio, affects photosynthetic activity, and the smaller this ratio, the lower the content of active cryptochrome 1. In this case, the difference between the WT and mutant with a deficit of cryptochromes decreases (first of all, likely cryptochrome 1). Indeed, the ratios of values of P_N, PI_{ABS}, and F_v/F_m at RL:BL ratio of 4:1 in WT to corresponding values in the mutant were lesser than these ratios at RL:BL:GL = 3:1:2. Also, the ratios of values of P_N and PI_{ABS} at in WT to corresponding parameters in the mutant were greater at RL:BL:GL = 3:1:1 ratio than those at RL:BL:GL = 4:1:0.3 ratio.

Previous work showed that the expression of genes of key antioxidant enzymes and enzymes related to the biosynthesis of enzymes of some low-molecular-mass antioxidants were higher in the WT than that in the *hy4* mutant grown at moderate BL (Kreslavski *et al.* 2021). These data and our results suggest that cryptochrome 1 is important for the regulation of expression of key antioxidant genes, and GL acting *via* cryptochrome 1 performs the function of adjusting a plant to shady conditions corresponding to a high GL/BL ratio.

Conclusion: Green light constitutes a significant portion of the spectrum of solar irradiation and there are a lot of data that demonstrate that GL modulates light-induced plant responses. The interaction of GL with other wavelengths provides a strategy for plant adaptation to changes in light environments.

The content of active cryptochrome 1 depends on GL/BL ratio and the higher the ratio, the smaller this

content. In this case, at a high fraction of GL in the spectrum, the WT can be similar to a mutant with a deficit of cryptochrome 1. Indeed, with added GL the parameters of photosynthetic activity such as PI_{ABS} and F_v/F_m in WT decreased and as a result a difference in the parameters between the WT and the mutant was lowered. Also, the pro-/antioxidant balance depends on the cryptochrome 1 deficit and BL/GL ratio. However, another situation can be in shadow leaves. Green light can enhance photosynthesis at the optimal contribution of GL to spectral emission.

An increase in the GL ratio in the light spectrum led to an increase in the expression of the main light-dependent transcription factors, as well as phytochrome D and phytochrome C, which indicates both the involvement of the transcription factors and the phytochromes in GL signaling and the threshold effect of GL.

Thus, cryptochrome 1 deficit reduces the photosynthetic rate and PSII activity, and antioxidant capacity in *Arabidopsis*. However, additional GL can partially remove these effects of cryptochrome 1.

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