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The resistance of *Solanum lycopersicum* photosynthetic apparatus to high-intensity blue light is determined mainly by the cryptochrome 1 content

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Abstract

The effects of deficiency of cryptochrome 1 (CRY1), phytochrome B2 (phyB2) and the photoreceptor signalling DET-1 protein (*hp-2* mutant) on photosynthesis and pro-/antioxidant balance in *Solanum lycopersicum* exposed to high-intensity blue light [HIBL, 72 h, 500/1,000 μmol(photon) m^{-2} s^{-1}] were studied. Noticeable photoinhibition of photosynthesis and PSII was found in all these variants. However, the greatest decrease in photosynthesis and PSII activity was observed in the *cry1* mutant. The difference among the other options was less pronounced. This low resistance of the *cry1* mutant to HIBL is associated with reduced photosynthetic pigments, phenols, and anthocyanins. It appears that under HIBL, CRY1 and, to a lesser extent, phyB2 are required to maintain photosynthesis and antioxidant defence, mitigating blue light-induced oxidative stress. This study expands our understanding of the defence functions of CRY1 and highlights its importance in adapting the photosynthetic apparatus to HIBL.

Keywords: blue irradiation; Chl *a* fluorescence; high irradiance; photoinhibition; photosynthetic activity; tomato.

Introduction

The influence of light quality on plant growth, photosynthesis, and photomorphogenesis is primarily mediated by the absorption of light quanta by specific photoreceptors and pigments. These photoreceptors include phytochromes, which detect red light and far-red light; cryptochromes (CRY), which are sensitive to blue

Highlights

- CRY1 is crucial for the adaptation of the photosynthetic apparatus to strong blue light
- Among all options, the *cry1* mutant shows the lowest carotenoid and phenolic contents
- The photosynthetic apparatus of the *hp-2* mutant is resistant to high-intensity blue light

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Abbreviations: BL – blue light; Chl – chlorophyll; CRY – cryptochromes; DI_0/RC – total energy dissipation per reaction centre; DM – dry mass; F_t – steady-state fluorescence in the light-adapted state; F_v/F_m – PSII maximal photochemical quantum yield; F_v' – variable fluorescence; g_s – stomatal conductance; HIBL – high-intensity blue light; hp – high pigment; NPQ – nonphotochemical quenching; PA – photosynthetic apparatus; PI_{ABS} – PSII performance index on an absorption basis; P_N – net photosynthetic rate; phyB – phytochrome B; SOD – superoxide dismutase; WT – wild type; $Y_{(II)}$ – PSII actual photochemical efficiency; $Y_{(NO)}$ – the quantum yield of PSII nonregulated nonphotochemical energy dissipation; $Y_{(NPQ)}$ – the quantum yield of PSII regulated nonphotochemical energy dissipation.

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light (BL) and ultraviolet-A; and phototropins, which also respond to BL and ultraviolet-A. These photoreceptors operate in specific spectral ranges, contributing to the coordination of growth, development, and stress responses in plants.

Cryptochromes are flavoproteins that contain the chromophore flavin adenine dinucleotide (FAD). The last plays the role of a reaction centre, and oxidized FAD is the original inactive form of the cryptochrome. After light absorption, FAD is reduced and protonated, forming the neutral, semi-reduced form of FADH*, which is the active form of the photoreceptor. This change initiates signalling pathways that activate transcription factors such as long hypocotyl 5 (HY5), a positive regulator of photomorphogenesis that promotes the expression of numerous light-responsive genes. These genes are involved in processes such as chloroplast biogenesis, pigment biosynthesis, stomatal regulation, and photoprotection (Chaves *et al.* 2011). Cryptochrome 1 (CRY1) stabilizes HY5 by inhibiting its degradation, which takes part in the COP1/SPA complex, which includes constitutive photomorphogenesis protein 1 (COP1) and suppressor of phytochrome A (SPA) (Jiao *et al*. 2003).

Phytochromes, particularly phytochrome B (PHYB), sense red and far-red light and play a central role in photomorphogenesis and photosynthesis regulation. The transition of PHYB from the inactive form to the active form leads to the degradation of phytochrome-interacting factors (PIFs), which are repressors of light-induced responses. This promotes the activation of light-responsive genes. Importantly, signalling pathways mediated by CRY1 and PHYB converge on shared targets such as HY5, integrating signals from blue and red light to generate coordinated plant responses (Su *et al*. 2017).

Under high-intensity light, plants face significant risks of photoinhibition, characterized by damage to PSII caused by excessive light energy exceeding the capacity of the photosynthetic apparatus to utilize it. CRY1 plays a central role in mitigating these effects by inducing the expression of early light-inducible proteins (ELIP1 and ELIP2), which stabilize PSII, and antioxidant enzymes such as ascorbate peroxidases, which detoxify reactive oxygen species (ROS) generated by high-intensity light (Kleine *et al*. 2007). The authors suggested a novel function of CRY1 in inducing photoprotective mechanisms in response to high irradiance. Additionally, CRY1 regulates the biosynthesis of pigments such as carotenoids and anthocyanins, which function as light-absorbing filters and antioxidants (Ashikhmin *et al.* 2023).

Despite significant progress, many aspects of light signalling and photoprotection remain poorly understood. Studies on *Arabidopsis thaliana* cryptochrome mutants have demonstrated that plants deficient in CRY1 show reduced contents of carotenoids, flavonoids, and anthocyanins under high blue light, leading to increased vulnerability to photoinhibition (Khudyakova *et al*. 2024). These findings underscore the importance of CRY1 in coordinating antioxidant defenses and maintaining photosynthetic efficiency under high-intensity light. However, how deficiencies in CRY1 and PHYB specifically affect the balance of pro- and antioxidants across different plant species is not yet fully understood. Moreover, the specific role of low-molecular-mass pigments, such as carotenoids and anthocyanins, in protecting the photosynthetic apparatus under high-intensity light in blue light conditions requires further investigation (Khudyakova *et al*. 2024).

The above results highlight the vulnerability of PSII to photoinhibition under high-light-intensity conditions. Photoinhibition, characterized by a decrease in photosynthetic efficiency due to high-intensity light, particularly affects PSII and involves the dynamic repair of damaged PSII sites through *de novo* biosynthesis of the D1 protein (Powles 1984, Foyer *et al*. 2017). Understanding the mechanisms by which high-intensity light influences PA and the pro-/antioxidant balance, particularly in the context of CRY1 deficiency, is critical, especially in high-intensity blue light (HIBL), where the role of CRY1 is most significant (Kleine *et al*. 2007).

Tomato mutants provide a valuable model for exploring the effects of photoreceptor deficiency on photoprotection and the development of oxidative stress. For example, mutants such as *high pigment 2* (*hp-2*) and *low pigment* (*lp*) indicate contrasting pigment contents, which influence the sensitivity of their photosynthetic apparatus to high-intensity light. Previous studies have shown that *hp-2* mutants, which carry a mutation in the DE-ETIOLATED1 gene, exhibit enhanced photoprotection under optimal growth conditions due to increased pigment accumulation and improved stomatal regulation. Conversely, *lp* mutants, characterized by low pigment content, are more susceptible to oxidative stress. These phenotypic differences provide an opportunity to investigate the interplay between photoreceptors, pigments, and photoprotection mechanisms under blue light conditions (Ashikhmin *et al*. 2024).

The present study aims to elucidate the mechanisms by which the photomorphogenetic proteins CRY1, PHYB, and the light-signalling component DE-ETIOLATED1 influence photosynthetic efficiency, pigment biosynthesis, and the balance of pro- and antioxidants during HIBL-induced oxidative stress. By focusing on tomato plants deficient in CRY1 and mutants with altered pigment contents, this research aims to deepen our understanding of how cryptochromes and phytochromes contribute to photoprotection and plant adaptation to extreme light conditions.

Materials and methods

Growth and irradiation conditions: Forty-five-day-old *Solanum lycopersicum* L. plants (Moneymaker, cultivar LA2706) of the wild type (WT) and photomorphogenic mutants (*hp-2*, LA3005, mutation of the de-etiolated 1 gene with elevated pigment content), and mutants with cryptochrome 1 deficiency (*cry1*, LA4359) and phytochrome B2 deficiency (*phyB2*, LA4358) were used. Seeds were obtained from the *Tomato Genetics Resource Center* (University of California, Davis, CA, USA). Note that the *hp-2* phenotype with de-etiolated 1 deficiency is light hypersensitive (Mustilli *et al*. 1999).

The plants were grown in a thermostated chamber with a 16-h photoperiod at a temperature of 26 ± 1°C during the day and 22 ± 1°C at night. The plants were subsequently cloned from cuttings kept in distilled water for 10 d under low-room light for rooting. After this, the plants were grown under white LEDs (Fig. 1) for 45 d in 8 × 8 × 10 cm vessels filled with perlite under a 12-h photoperiod and an intensity of 200 µmol(photon) m^{-2} s^{-1} under the above temperature conditions. During the cultivation period, the plants were watered with half Hoagland's solution. After growth, the plants were continuously irradiated with high-intensity blue light for 72 h using LEDs [intensity = 500 ± 20 and 1,000 ± 50 µmol(photon) m^{-2} s^{-1}, λ_{max} = 457 nm, half-width = 26 nm]. The spectral characteristics of the light sources (Fig. 1) were determined using a "*MK350N Premium*" spectroradiometer (Zhunan Township, Taiwan). The five to ten most developed leaves of the third and second tiers were used for analysis.

CO_2 gas exchange and fluorescence parameters of chlorophyll: The net photosynthetic rate (P_N) was evaluated by a closed device using a portable *LCPro+* infrared gas analyser (*ADC BioScientific Ltd.*, Hoddesdon, UK). This analyser was connected to the leaf chamber, with a 6.25 cm^2 area. The photosynthetic rate in the leaves was evaluated at a light intensity of 1,000 µmol(photon) m^{-2} s^{-1}.

The fluorescence induction curves were obtained with a *mini-PAM II* fluorometer (*Walz*, Effeltrich, Germany). The plants were allowed to adapt to the dark for 20 min. Blue LEDs (λ_{max} = 474 nm) were used to generate actinic light [200 µmol(photon) m^{-2} s^{-1}] and saturating light pulses [λ_{max} = 474 nm; 5,000 µmol(photon) m^{-2} s^{-1}; and 800 ms duration].

The values of F_0, F_v, F_m, F_m', and F_0', as well as the PSII maximum (F_v/F_m) and effective $Y_{(II)}$ photochemical quantum yields and the quantum yields of PSII nonregulated [$Y_{(NO)}$] and regulated [$Y_{(NPQ)}$] nonphotochemical energy dissipation, were determined (Kramer *et al*. 2004, Schreiber 2004, Klughammer and Schreiber 2008). Also, nonphotochemical fluorescence quenching NPQ was determined according to the following equation: $NPQ = (F_m - F_m')/F_m'$, and $Y_{(II)}$ was determined according to the equation $Y_{(II)} = (F_m' - F_t)/F_m$. Here, F_m and F_m' are the maximum Chl fluorescence levels under dark- and light-adapted conditions, respectively. F_v is the photo-induced change in fluorescence, F_t is the steady-state level of Chl fluorescence, and F_0 and F_0' are the initial Chl fluorescence levels under dark- and light-adapted conditions. In addition, fluorescence parameters were estimated based on the JIP test by the fluorimeter described in Kreslavski *et al*. (2014). Here, the induction curves were measured using blue light [maximum – 455 nm and 5,000 µmol(photon) m^{-2} s^{-1}]. Computer-collected data were obtained under the following conditions: the signal was received every 10 µs for 1 ms and every 1 ms from 1 ms to 1 s. The values F_0, F_v, and F_m were recorded, where F_m and F_0 are the maximum and minimum Chl *a* fluorescence amplitudes under dark-adaptation conditions, respectively, and F_v is the photoinduced change in Chl *a* fluorescence.

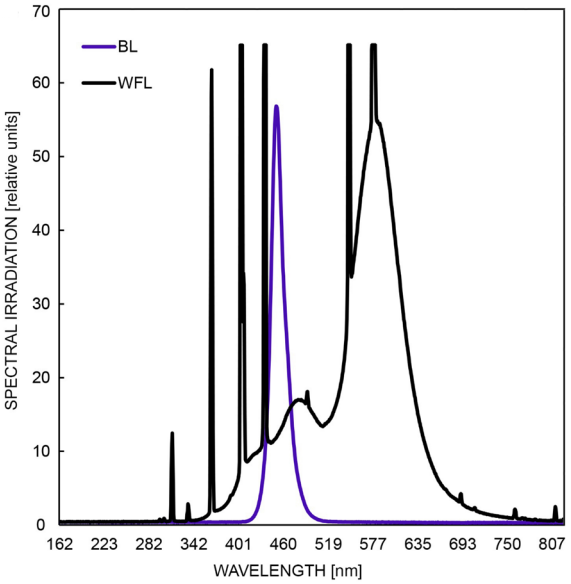

Fig. 1. Spectra of the light sources used in the plant growth experiments: BL – blue light, WFL – white fluorescent light.

The recorded induction curves were used for calculations of the maximal quantum yield F_v/F_m, the DI_0/RC parameter, which is an indicator of the total energy dissipated by one active reaction centre, mainly in the form of heat, and PI_{ABS}, which is the PSII performance index (Goltsev *et al*. 2016, Kalaji *et al*. 2016).

Photosynthetic pigments, phenols, and anthocyanins: The content of carotenoids was measured in 96% ethanol extracts (Lichtenthaler 1987) *via* analysis of the absorption spectra by a *Genesys 10 UV* spectrophotometer (*Thermo Fisher Scientific*, Waltham, MA, USA) at λ_{max} values of 470, 649, and 665 nm.

The total phenolic content was determined spectrophotometrically with Folin and Ciocalteu's phenol reagents (*Sigma-Aldrich*, Burlington, MA, USA, MDL number MFCD00132625) according to a previously described method (Singleton and Rossi 1965). The total phenolic content was expressed in gallic acid equivalents (GAE) in mg g^{-1}[fresh mass (FM) or dry mass (DM)].

Anthocyanins were determined spectrophotometrically (Liu *et al*. 2018). The leaf mass (0.10–0.15 g) per sample was ground in liquid nitrogen and incubated in 600 µl of extraction buffer (methanol containing 1% HCl) in an ultrasonic bath for 15 min and then overnight at 4°C in the dark. Furthermore, the samples were centrifuged for 5 min at 10,000 × *g*. Then, the supernatant was transferred to a 2-mL centrifuge tube, 400 µl of water and 400 µl of chloroform were added to each sample and vortexed, followed by centrifugation at 10,000 × *g* and 4°C for 5 min. The absorbance of the supernatant was determined at wavelengths of 530 (A_{530}) and 657 (A_{657}) nm. The anthocyanin content was calculated as $A_{530} - 0.33 \times A_{657}$ g^{-1}(FM).

Content of malondialdehyde and antioxidant enzyme activity: The degree of lipid peroxidation was determined

by measuring the content of malondialdehyde (MDA) according to Zhang *et al*. (2007). Fresh leaf tissue (0.5 g) was homogenized in 5 ml of 5% trichloroacetic acid (TCA) and centrifuged at 5,000 × *g* for 20 min at 4°C. Then, 1.5 ml of the supernatant was added to 2.5 ml of a reaction mixture containing 20% trichloroacetic acid and 0.5% thiobarbituric acid. The mixture was kept at 100°C for 25 min, after which it was cooled at room temperature and centrifuged at 5,000 × *g* for 10 min. Absorbance was measured on a *Genesis 10 UV* spectrophotometer (*Spectronic Unicam*, USA) at 532 nm. The value was corrected for nonspecific absorbance at 600 nm. The concentration of malondialdehyde was calculated using an extinction coefficient of 155 μmol^{-1} cm^{-1} and expressed as $\mu mol\ g^{-1}$(FM).

Superoxide dismutase (SOD) activity was determined by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium at 560 nm according to Gupta *et al*. (1993) with minor modifications. The activity of guaiacol-dependent peroxidase (POD) was evaluated by the method described in Maehly and Chance (1954). Enzyme activity was measured by an increase in absorbance at 470 nm (ε = 26.6 mM^{-1} cm^{-1}) compared with that of the controls without the enzyme. The specific enzyme activity was estimated based on fresh biomass.

Statistics: Fluorescence measurements and photosynthetic and transpiration rate measurements were carried out in four biological replicates on fully developed leaves of the third and second tiers. For each variant, an average of eight leaves from four plants were used. The significance of differences between various variances was calculated by one-way analysis of variance (*ANOVA*) based on *Duncan*'s method *via SigmaPlot 12.3* (*Systat Software Inc.*, San Jose, USA). The letters above the columns indicate significant differences between the options ($p<0.05$). The data are presented as arithmetic means ± SDs.

Results

Fluorescence parameters, photosynthetic and transpiration rates, and stomatal conductance: Initially, the parameters reflecting PSII activity were slightly different among all options (Table 1, Fig. 2*A*,*B*). The P_N of the *cry1* and *hp-2* mutants was lower than that of the WT. The g_s was the highest in the *cry1* mutant (Table 2).

Irradiation of any plants with high BL led to a decrease in PSII activity (F_v/F_m, PI_{ABS}, and $Y_{(II)}$) and a decrease in P_N and g_s but an increase in DI_0/RC (Tables 1, 2; Fig. 2*A*,*B*).

At 24 h and 500 µmol(photon) m^{-2} s^{-1}, PSII activity was greater in the *hp-2* mutant than in the *cry1* mutant. Here, P_N was the highest in the WT and lowest in the *cry1* mutant. At 72 h and 500 µmol(photon) m^{-2} s^{-1}, significant differences in PSII activity between the variants were not detected, excluding the *cry1* mutant, where this activity was the lowest. The P_N value was the highest in the WT and *hp-2* mutant and the lowest in the *cry1* mutant.

At 24 h and 1,000 µmol(photon) m^{-2} s^{-1}, PSII activity was highest in the WT, and the lowest activity was detected in the *cry1* and *hp-2* mutants. P_N was the greatest in the WT.

Table 1. Impact of blue light irradiance dose on fluorescent parameters (F_v/F_m, PI_{ABS}, DI_0/RC) and the net photosynthetic rate (P_N) in 45-day-old wild-type (WT), *cry1*, *hp-2*, and *phyB2* mutant tomato plants exposed for 24 h and 72 h to light intensity of 500 or 1,000 µmol(photon) m^{-2} s^{-1}. The mean values ± SDs are shown. *Different letters* indicate a significant difference at $p<0.05$, $n = 4$.

Mutant/time [h]	Light intensity [µmol (photon) m^{-2} s^{-1}]	F_v/F_m	PI_{ABS}	DI_0/RC	P_N [µmol(CO_2) m^{-2} s^{-1}]
WT 0 h	0	0.82 ± 0.01^{a}	6.23 ± 0.54^{ab}	0.42 ± 0.02^{c}	7.8 ± 0.4^{a}
cry1 0 h	0	0.81 ± 0.01^{a}	4.91 ± 0.51^{b}	0.44 ± 0.02^{c}	6.4 ± 0.2^{b}
hp-2 0 h	0	0.82 ± 0.01^{a}	6.29 ± 0.18^{a}	0.43 ± 0.01^{c}	6.6 ± 0.3^{ab}
phyB2 0 h	0	0.83 ± 0.01^{a}	6.71 ± 0.28^{a}	0.40 ± 0.03^{c}	6.8 ± 0.2^{ab}
WT 24 h	500	0.56 ± 0.03^{c}	0.62 ± 0.11^{cd}	1.34 ± 0.38^{ab}	5.9 ± 0.3^{b}
cry1 24 h	500	0.54 ± 0.04^{bc}	0.24 ± 0.07^{e}	1.93 ± 0.35^{a}	4.2 ± 0.2^{d}
hp-2 24 h	500	0.66 ± 0.02^{b}	0.97 ± 0.14^{c}	0.70 ± 0.15^{b}	5.1 ± 0.1^{c}
phyB2 24 h	500	0.63 ± 0.05^{bc}	0.84 ± 0.14^{cd}	0.98 ± 0.21^{b}	5.2 ± 0.3^{bc}
WT 72 h	500	0.57 ± 0.01^{c}	0.56 ± 0.04^{d}	1.23 ± 0.20^{ab}	4.9 ± 0.4^{bc}
cry1 72 h	500	0.45 ± 0.03^{d}	0.31 ± 0.01^{e}	2.19 ± 0.35^{a}	3.0 ± 0.3^{e}
hp-2 72 h	500	0.56 ± 0.03^{c}	0.47 ± 0.04^{d}	1.52 ± 0.26^{ab}	5.3 ± 0.1^{bc}
phyB2 72 h	500	0.60 ± 0.03^{bc}	0.57 ± 0.02^{d}	1.61 ± 0.20^{ab}	4.6 ± 0.4^{cd}
WT 24 h	1,000	0.57 ± 0.04^{b}	0.57 ± 0.08^{d}	1.24 ± 0.30^{c}	6.0 ± 0.4^{b}
cry1 24 h	1,000	0.34 ± 0.05^{c}	0.10 ± 0.01^{f}	3.75 ± 0.45^{b}	3.2 ± 0.1^{d}
hp-2 24 h	1,000	0.34 ± 0.04^{c}	0.18 ± 0.04^{ef}	4.23 ± 0.92^{b}	3.6 ± 0.1^{d}
phyB2 24 h	1,000	0.42 ± 0.04^{c}	0.23 ± 0.01^{e}	2.25 ± 0.32^{b}	4.3 ± 0.2^{c}
WT 72 h	1,000	0.53 ± 0.02^{b}	0.68 ± 0.11^{cd}	1.49 ± 0.06^{c}	3.5 ± 0.2^{d}
cry1 72 h	1,000	0.04 ± 0.01^{d}	0.02 ± 0.01^{g}	1.04 ± 0.15^{a}	0.4 ± 0.2^{e}
hp-2 72 h	1,000	0.54 ± 0.03^{b}	0.48 ± 0.10^{d}	2.23 ± 0.28^{b}	4.3 ± 0.2^{c}
phyB2 72 h	1,000	0.58 ± 0.02^{b}	0.96 ± 0.08^{c}	0.87 ± 0.04^{d}	4.0 ± 0.4^{cd}

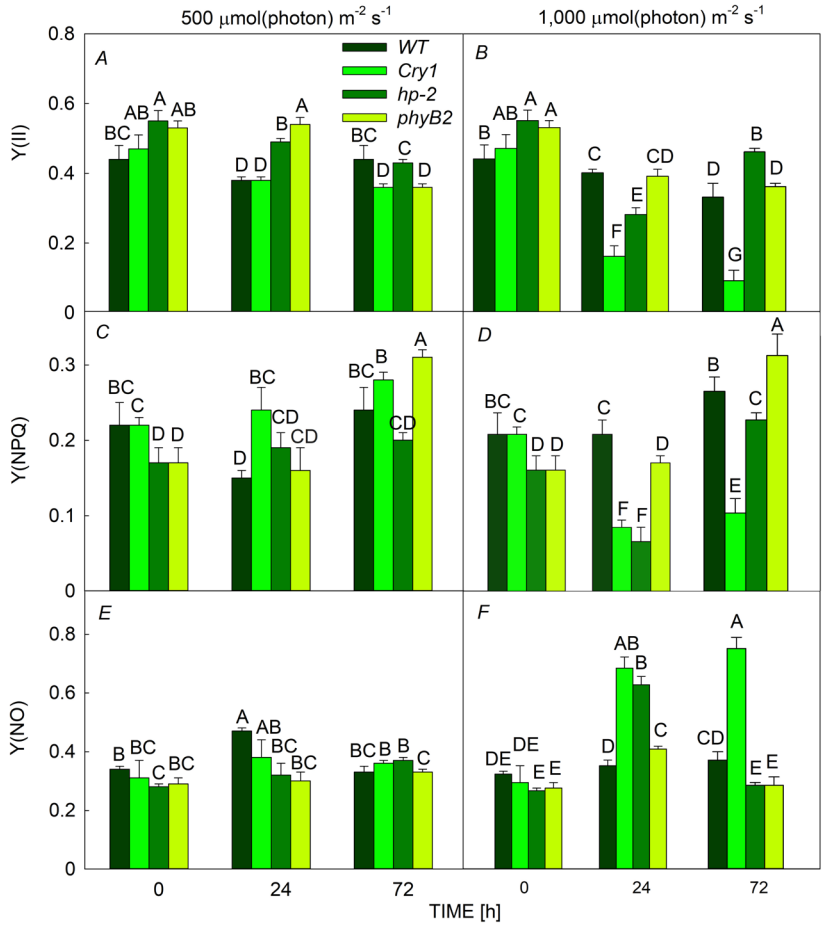

Fig. 2. Impact of blue light dose on the fluorescence parameters of $Y_{(II)}$ (*A*,*B*), $Y_{(NPQ)}$ (*C*,*D*), and $Y_{(NO)}$ (*E*,*F*) in 45-day-old wild-type (WT), *cry1*, *hp-2*, and *phyB2* mutant tomato plants exposed for 24 h and 72 h at intensities of 500 and 1,000 μmol(photon) m^{-2} s^{-1}. Here, $Y_{(II)}$ is the effective photochemical quantum yield, and $Y_{(NPQ)}$ and $Y_{(NO)}$ are the quantum yields of PSII nonregulated and regulated nonphotochemical energy dissipation, respectively. The mean values ± SDs are shown. *Different letters* at each light intensity indicate a significant difference at $p<0.05$, $n = 4$.

Table 2. Impact of blue light irradiance dose on stomatal conductance g_s [mmol m^{-2} s^{-1}] in 45-day-old wild type (WT), *cry1*, *hp-2*, and *phyB2* mutant tomato plants exposed for 72 h to light intensity of 500 and 1,000 μmol(photon) m^{-2} s^{-1}. The mean values ± SDs are shown. *Different letters* at each light intensity indicate a significant difference at $p<0.05$, $n = 4$.

Mutant/time [h]	Light intensity [μmol(photon) m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]
WT 0 h	0	73 ± 4^{c}
cry1 0 h	0	121 ± 5^{a}
hp-2 0 h	0	84 ± 3^{bc}
phyB2 0 h	0	88 ± 3^{b}
WT 72 h	500	21 ± 2^{d}
cry1 72 h	500	10 ± 1^{b}
hp-2 72 h	500	12 ± 2^{e}
phyB2 72 h	500	16 ± 2^{de}
WT 72 h	1,000	17 ± 3^{de}
cry1 72 h	1,000	9 ± 1^{e}
hp-2 72 h	1,000	12 ± 1^{e}
phyB2 72 h	1,000	15 ± 3^{de}

At 72 h and 1,000 μmol(photon) m^{-2} s^{-1}, all parameters, F_v/F_m, PI_{ABS}, $Y_{(II)}$, and P_N, were the lowest in the *cry1* mutant. P_N was greater in the *hp-2* mutant than in the other treatment groups.

The g_s decreased in all the treatment groups, but the greatest decrease in g_s was detected in the *cry1* mutant at both light intensities (Table 2).

Under all light treatments, the PSII activity or P_N of the *phyB2* mutant was comparable to that of the corresponding WT plants.

In addition, the changes under the different treatments were opposite to those in PSII activity. The lower the activity was the greater the value of DI_0/RC (Table 1).

The greatest difference in $Y_{(NPQ)}$ and $Y_{(NO)}$ values was found at 1,000 μmol(photon) m^{-2} s^{-1} and 72 h among the *cry1* mutant and all other variants (Fig. 2*C–F*). Thus, at 72 h, in this mutant, the $Y_{(NPQ)}$ value was 2–3 times lower, and the $Y_{(NO)}$ value was two or more times greater than those in all other options. At 1,000 μmol(photon) m^{-2} s^{-1} and 24 h, the $Y_{(NPQ)}$ values in *cry1* and *hp-2* were at least two times lower than those in the WT and *phyB2* mutant. Conversely, the $Y_{(NO)}$ values were almost twice as high as the corresponding values in the WT and *phyB2* mutant.

Carotenoid, phenolic, and anthocyanin contents: Initially, the contents of carotenoids and phenols were greater in the *hp-2* mutant than in the WT and other mutants (Fig. 3*A–D*). The content of carotenoids, phenols, and anthocyanins (Fig. 3*E*,*F*) in the *cry1* mutant plants under all light and time conditions (0, 24, and 72 h) were lower than those in the WT, *hp-2*, and *phyB2* mutant plants. However, in all the variants, the phenolic content

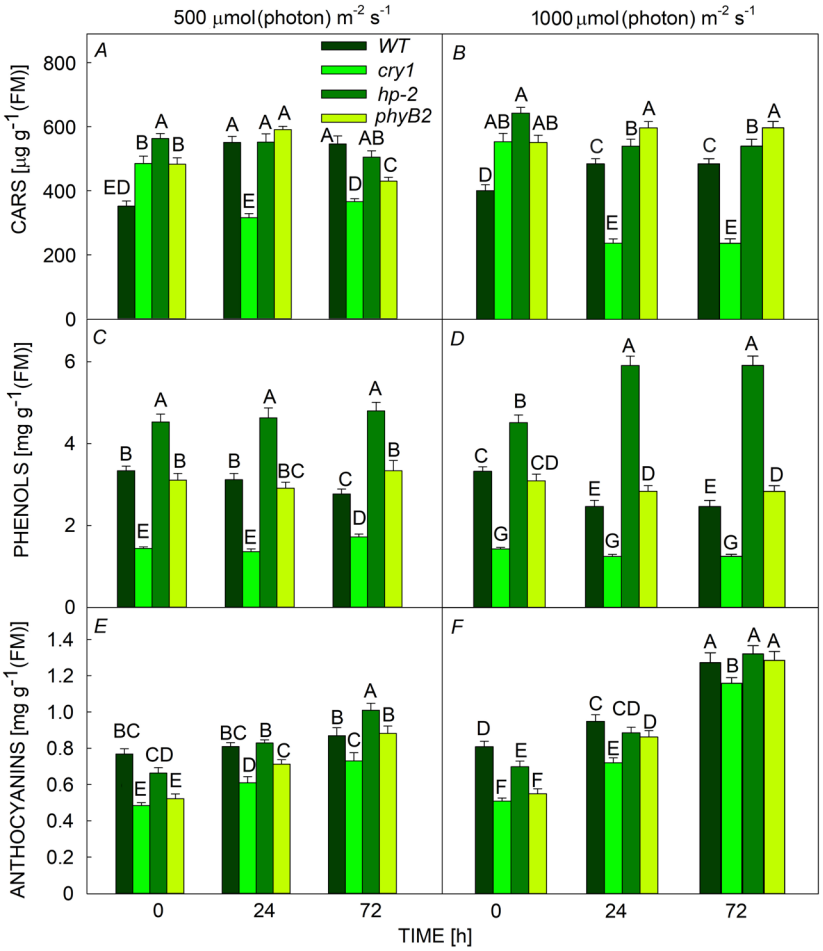

Fig. 3. Impact of blue light irradiation on pigment contents: carotenoids (Car) (*A*,*B*), phenols (*C*,*D*), and anthocyanins (*E*,*F*) in 45-day-old wild-type (WT), *cry1*, *hp-2*, and *phyB2* mutant tomato plants. The plants were exposed for 24 h and 72 h at light intensities of 500 and 1,000 μmol (photon) m^{-2} s^{-1}. The mean values ± SDs are shown. *Different letters* at each light intensity indicate a significant difference at $p<0.05$, $n = 4$.

was the highest in the *hp-2* mutant (Fig. 3*C*,*D*). Thus, the content of phenols in the *hp-2* mutant after 72 h of irradiation was 5.92 mg g^{-1}(FM) compared with 1.26 mg g^{-1}(FM) in the *cry1* mutant. Additionally, at 500 μmol(photon) m^{-2} s^{-1} and 72 h of BL exposure, the highest content of anthocyanins was detected in the *hp-2* mutant [1.01 mg g^{-1}(FM)] (Fig. 3*E*).

Malondialdehyde content and antioxidant enzyme activity: A significant increase in guaiacol-dependent peroxidase activity was detected in the *hp-2* mutant (Fig. 4*A*). The guaiacol-dependent peroxidase activity in the other treatment groups decreased or remained constant. SOD activity decreased or did not change (Fig. 4*B*). Additionally, it was the highest in the *cry1* mutant. The malondialdehyde content increased in all the mutants at light intensities of 500 and 1,000 μmol(photon) m^{-2} s^{-1} (Fig. 4*C*). In particular, a significant increase was detected in the *hp-2* mutant, where after 24 and 72 h of exposure to HIBL, the malondialdehyde content increased from 76.7 to 107 mmol g^{-1}(FM), and then to 115.6 mmol g^{-1}(FM), respectively.

Discussion

The regulation of light signal transduction components and the contents of photoreceptors may be an effective approach for modifying nutritional quality, especially pigment biosynthesis in leaves and, ultimately, in tomato fruits (Liu *et al*. 2004, Levin *et al*. 2006, Ashikhmin *et al*. 2024).

Carotenoids, particularly lycopene, are vital antioxidants in tomato plants and play a central role in mitigating oxidative stress and improving fruit nutritional quality (Liu *et al*. 2004). Previous studies have demonstrated that tomato *hp* mutants, characterized by high pigment accumulation, increase the production of flavonoids and photosynthetic pigments in leaves under physiological conditions (Liu *et al*. 2004, Levin *et al*. 2006, Ashikhmin *et al*. 2023, 2024). The authors concluded that the increased content of pigments such as phenolic compounds and carotenoids in the *hp-2* mutant is critical for the adaptation of the tomato photosynthetic apparatus to moderate doses of HIBL, whereas the content of CRY1 becomes more important at higher doses (Ashikhmin *et al*. 2024). However, under these conditions, changes in light quality did not lead to photoinhibition or the development of oxidative stress.

Understanding the role of pigments and photoreceptors in protecting the photosynthetic apparatus under high irradiance-induced oxidative stress is critical for comprehending plant adaptive mechanisms. High-intensity blue light (HIBL) provides a controlled environment to study these stress responses, allowing us to isolate the specific contribution of CRY1 to photoprotection. Our

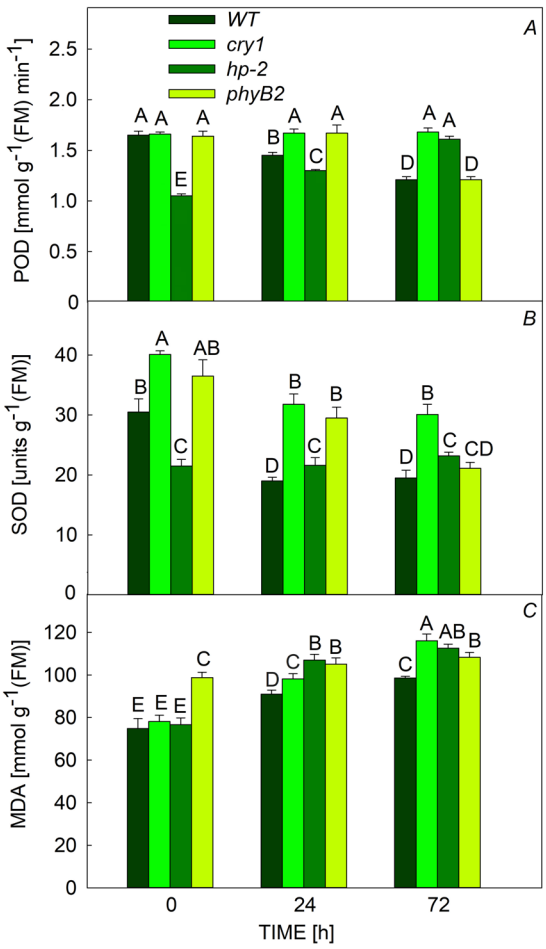

Fig. 4. Impact of blue light irradiation on guaiacol-dependent peroxidase (POD) (*A*) and superoxide dismutase (SOD) (*B*) activity and malondialdehyde (MDA) content (*C*) in 45-day-old wild-type (WT), *cry1*, *hp-2*, and *phyB2* mutant tomato plants. The plants were exposed to an intensity of 1,000 µmol(photon) m^{-2} s^{-1} for 24 h or 72 h. The mean values ± SDs are shown. *Different letters* indicate a significant difference at $p<0.05$, $n = 4$.

study employed HIBL as a model to examine the function of CRY1 in regulating the antioxidant and photoprotective responses of tomato plants.

Mutants deficient in CRY1 (*cry1*), as well as those with increased pigment contents, such as *hp-2* (high pigment) and deficient in PHYB2 (*phyB2*), were used to investigate the interplay between photoreceptors and pigments under oxidative stress. Previous findings revealed that the increased accumulation of pigments such as phenolic compounds and carotenoids in the *hp-2* mutant enhances the resilience of the photosynthetic apparatus to moderate doses of HIBL, supporting its ability to neutralise reactive oxygen species (Ashikhmin *et al*. 2023). Conversely, under higher light intensities, CRY1 becomes a more critical factor, contributing to the stabilisation of PSII and the regulation of nonphotochemical quenching (Ashikhmin *et al*. 2024).

Since we were interested in the behaviour of these mutants under light stress conditions, we selected tomato growth conditions such that when plants were exposed to HIBL, noticeable photoinhibition was observed. For this purpose, during plant growth, we used an emission spectrum with a small fraction of BL (approximately 10%) (Fig. 1).

Fluorescence parameters such as the PSII maximum (F_v/F_m) and effective [$Y_{(II)}$] photochemical quantum yields, which characterise the potential efficiency of PSII and its efficiency under light conditions, respectively, are used to evaluate PSII activity. Together with the value of the performance index (PI_{ABS}), these parameters are usually used to evaluate the damage of the PA during the development of oxidative stress (Goltsev *et al*. 2016).

All parameters, such as the F_v/F_m, $Y_{(II)}$, and PI_{ABS}, significantly decreased during light irradiation in all options. However, the most significant decline was in the value of the performance index (PI_{ABS}), which was the most sensitive to strong blue light. On the other hand, we observed a noticeable increase in the DI_0/RC ratio, indicating that some of the absorbed light energy dissipated per reaction centre, mainly as heat (Goltsev *et al*. 2016). The last process is one way to utilise excess energy and is most noticeable in the *cry1* mutant. Additionally, a noticeable decrease in the net photosynthetic rate and stomatal conductance was observed, which correlated with an increase in the content of malondialdehyde, especially in the *cry1* mutant, as a result of exposure to HIBL (Fig. 1). This indicates a state of chronic photoinhibition, which leads to direct damage to the PA, especially PSII (Zavafer and Mancilla 2021). This damage is associated with chemical changes in PSII and requires the restoration of PSII and the *de novo* synthesis of protein D1 (Aro *et al*. 1993, Tyystjärvi 2013, Bhutta *et al*. 2023). Understanding the extent to which the state of photoreceptors and light-signalling components such as de-etiolated 1 influence the adaptation of plants to HIBL, leading to the development of oxidative stress, is important for understanding the mechanism of PA adaptation.

A comparison of the photosynthetic parameters under HIBL conditions revealed that at 500 and 1,000 µmol(photon) m^{-2} s^{-1} and 72 h of irradiation, the PSII activity and photosynthetic rate were the lowest in the *cry1* mutant (Table 1, Fig. 2*A*,*B*). Moreover, at an intensity of 1,000 µmol(photon) m^{-2} s^{-1}, only the PA of the *cry1* mutant was significantly damaged.

Thus, CRY1, likely in its active form, is a critical regulator of the sensitivity of the PA to HIBL. This is supported by our findings, which align with previous work (Ashikhmin *et al*. 2024) showing that WT tomato plants, as well as *hp-2* and *lp* mutants, presented an increased rate of photosynthesis when grown under white fluorescent light and subsequently exposed to intense blue light. Conversely, the *cry1* mutant failed to exhibit this increase in photosynthetic activity, demonstrating that CRY1 is essential for maintaining photosynthetic efficiency under high-intensity light. These findings suggest that CRY1 mediates critical protective responses, particularly under elevated oxidative stress induced by HIBL.

According to our data, after 72 h of irradiation at 1,000 µmol(photon) m^{-2} s^{-1}, the P_N in the WT decreased

by 2.2 times, and in the *hp-2* mutant, the P_N decreased by 1.5 times (Table 1). These findings suggest that the *hp-2* mutant has greater adaptive potential than the WT does, likely due to its greater phenolic content and increased guaiacol-dependent peroxidase activity. Thus, we found that the *hp-2* mutant with increased pigment content retained photosynthetic rates at high doses of HIBL, comparable to those of the WT. This result is not consistent with data on the increased photosensitivity of *hp* mutants (Levin *et al*. 2006). This can be explained by the increased content of various pigments, particularly carotenoids and flavonoids, which can play a role in the adaptation of tomato PA to intense blue light (Ashikhmin *et al*. 2024). Additionally, no photoinhibition of the *hp-2* mutant in that study was detected, in contrast to that of the WT plants exposed to high-intensity white light [2,000 μmol(photon) m^{-2} s^{-1}]. Those authors explained the increased content of pigments and low-molecular-mass antioxidants in the *hp-2* mutant. In our case, elevated photosensitivity was characterised by a high content of malondialdehyde in the mutant, but the ability of the PA to adapt was supported by an increased content of pigments under these conditions.

Apparently, PhyB2 deficiency did not have much effect on PA adaptation to either short-term (24 h) or long-term (72 h) HIBL exposure, regardless of light intensity (Table 1). This may be due to the significantly lower phyB2 content under blue light than under red light (Kreslavski *et al*. 2018), which does not have a significant effect on the PA response to high-intensity light.

The induction of various pigments, such as anthocyanins and carotenoids, appears to be a way of protecting the PA from oxidative stress caused by excess light (Stetsenko *et al*. 2020). In plants, these pigments are protective compounds that act as optical filters or antioxidants, protecting components of the photosynthetic electron transport chain from excess light (Solovchenko and Merzlyak 2008, Solovchenko 2010). This is especially important when the incident light spectrum contains a significant proportion of BL, which is effectively absorbed by carotenoids and anthocyanins. The content of these pigments was the lowest in the *cry1* mutant, which can explain its low resistance to HIBL (Fig. 3*A*,*B*,*E*,*F*). In addition, the g_s value decreased the most significantly in the *cry1* mutant. Both factors play decisive roles in the low resistance of this mutant to HIBL.

Based on a study of the rate of photosynthesis and PSII photochemical activity measured in tomato photoreceptor mutants deficient in various photoreceptors, Abramova *et al.* (2023) concluded that CRY1 and phyB2 are key photoreceptors involved in the adaptation of the PA to UV-B. Our data on the effects of HIBL on tomato PA indicate that pigment and phyB2 contents opposite those of CRY1 play a less important role in adaptation to HIBL. Moreover, according to our data, CRY1 plays a key role if the intensity of the BL in the spectrum is quite high.

One of the mechanisms for reducing the negative effect of oxidative stress during photoinhibition is the dissipation of absorbed light energy into heat, characterised by DI_0/RC values (Ruban 2016). High values of this parameter indicate the effectiveness of this dissipation, suggesting efficient dissipation of light energy. This pathway works most efficiently in the *cry1* mutant. Similarly, dissipation into heat through various channels is characterised by quantum yields $Y_{(NPQ)}$ and $Y_{(NO)}$. We hypothesise that non-light-induced, unregulated fluorescence quenching $Y_{(NO)}$ is most pronounced in the *cry1* mutant, playing a role in its adaptation to the highest dose of BL.

The high-intensity blue light (HIBL) conditions used in this study, while artificial compared with the natural environment of tomato plants, were specifically chosen to isolate the role of cryptochrome 1 (CRY1) and its interaction with the photosynthetic apparatus under extreme light stress. HIBL amplifies photoreceptor activity, enabling a focused analysis of photoprotection mechanisms, including nonphotochemical quenching, antioxidant enzyme activity (*e.g*., ascorbate peroxidase), and pigment biosynthesis (carotenoids, anthocyanins). These mechanisms are often masked under fluctuating natural light. Although nonphysiological, these conditions provide insight into plant adaptation to oxidative stress and highlight the role of CRY1 in modulating gene networks involved in photosynthetic regulation and photoinhibition repair, which are relevant for stress-resistant crop development.

Conclusion: We suggest that the ability of the tomato photosynthetic apparatus to adapt to HIBL depends primarily on the content of the active form of CRY1 and, to a lesser extent, on de-etiolated 1 deficiency, which leads to an increase in the number of carotenoids and phenolic compounds in leaves and phyB2 deficiency. The influence of these factors depends on the light conditions under which the plants were grown before exposure to high-intensity blue light, particularly the fraction of blue light and UV in the spectrum. Importantly, the *hp-2* mutant with increased pigment content retained this ability even under conditions of strong photoinhibition caused by HIBL and adapted to it. The effective formation of the active form of CRY1 is necessary for the sustainability of the photosynthetic apparatus to high-intensity light, which requires the presence of a sufficiently high-intensity blue spectral component in the initial light. CRY1 is a promising target for producing plants with increased resistance to photoinhibition under field conditions. However, further research is needed to understand the role of different photoreceptors and signalling components, as well as their application to improve nutritional quality and adaptation potential under high light conditions. For example, blue light inhibitors of cryptochromes 1 and 2 (BIC1 and BIC2) can inhibit cryptochrome function, blocking blue light-induced cryptochrome dimerization (Wang *et al*. 2017, Ponnu and Hoecker 2022). However, how cryptochromes regulate BIC gene activity remains unclear. Recently, some studies have shown that cryptochromes take part in the light-induced activation of BIC gene transcription. Here, cryptochromes suppress the activity of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), which leads to the activation of the transcription activator HY5, which is linked to chromatins of the BIC promoters. Additionally, phytochrome can induce light activation of BIC transcription.

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