PHOTOSYNTHETICA
International Journal for Photosynthesis Research

DOI 10.32615/ps.2022.036 PHOTOSYNTHETICA 61 (SI): 138-147, 2023

Special issue on Recent advances in photomodulation in higher plants, algae, and bryophytes

Influence of phytochromes on microRNA expression, phenotype, and photosynthetic activity in *A. thaliana phy* mutants under light with different spectral composition

P. PASHKOVSKIY[*,+], V. KRESLAVSKI[**], A. KHUDYAKOVA[**], A. KOSOBRYUKHOV[**], Vl.V. KUZNETSOV[*], and S.I. ALLAKHVERDIEV[*,+]

K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, 127276 Moscow, Russia[*]
Institute of Basic Biological Problems, Russian Academy of Sciences, Institutskaya Street 2, Pushchino, 142290 Moscow Region, Russia[**]

Abstract

Light-induced changes in miRNAs, morphogenesis, and photosynthetic processes in phytochrome-deficient mutant plants grown under different light qualities were studied. miRNA activity in many processes is regulated by phytochromes and phytochrome-interacting factors (PIFs). The reduced content of photoreceptors in phytochrome mutants affects the PIF–microRNA interaction. In plants grown under red light (RL) and white light (WL), the phenotype of *phyb* mutant was distorted; however, under blue light (BL) conditions, the *phyb* phenotype was normalized. The photosynthetic rates of both the mutants and wild type were higher under BL than under RL and WL. The expression of most studied miRNAs increased in *phyaphyb* mutants under BL conditions, which is probably one of the reasons for the normalization of the phenotype, the increase in PSII activity, and the photosynthetic rate. MicroRNAs under BL can partially improve photosynthesis and phenotype of the mutants, which indicates the conjugation of the functioning of phytochromes in miRNA formation.

Keywords: *Arabidopsis thaliana* phytochrome mutants; microRNA; photomorphogenesis; photosynthesis.

Introduction

Light is a key environmental factor affecting plant growth and development. To adapt to changing conditions, plants perceive light signals through the following groups of specific photoreceptors (Kong and Okajima 2016, Su *et al*. 2017, Voitsekhovskaja 2019): phytochromes for red light (Quail 2010); phototropin (Phot1–2) (Sullivan and

Highlights

- Blue light increases the photosynthetic activity of phytochrome mutants and normalises their phenotype
- Blue light leads to an increase in the expression of light-dependent miRNAs
- Light-dependent miRNAs partially determine the normalization of the phenotype

Received 28 June 2022
Accepted 28 July 2022
Published online 25 August 2022

[+]Corresponding author
e-mail: pashkovskiy.pavel@gmail.com
suleyman.allakhverdiev@gmail.com

Abbreviations: BL – blue light; CRY– cryptochrome; HY5 – elongated hypocotyl 5; miR, miRNA – mature microRNA; MIR – microRNA gene; PA – photosynthetic apparatus; PHY – phytochrome; RL – red light; TF – transcription factor; WL – white light.

Acknowledgements: This work and Fig. 1S (*supplement*) were financially supported by the Ministry of Science and Higher Education of the Russian Federation No. 122042700044-6 and No. 122050400128-1, respectively. Data of photosynthetic rates and fluorescent parameters were obtained with the financial support of the Ministry of Science and Higher Education of the Russian Federation (No. 122041100071-1, 122050400128-1), with partial financial support from the Russian Foundation for Basic Research (No. 20-04-00512).

Conflict of interest: The authors declare that they have no conflict of interest.

Deng 2003) and cryptochrome (Cry1–3) (Chaves *et al.* 2011) for UV-A and blue light; and UVR8 for UV-B light (280–315 nm) (Hayes *et al.* 2014). Phytochromes are photoreceptors that perceive and respond to light in the red and far red regions of the spectrum. Five genes encoding phytochrome apoproteins (*PHYA–PHYE*) have been identified in the *A. thaliana* genome, with *PHYA* and *PHYB* being the most important (Quail 2010, Kreslavski *et al.* 2018, Voitsekhovskaja 2019). It is known that the light-signalling system includes photoreceptors, including phytochromes and light signal-transduction components, and regulates plant growth and development processes, such as germination, photoperiodic response, plant stress resistance, and chlorophyll (Chl) biosynthesis (Möglich *et al.* 2010, Kreslavski *et al.* 2018, 2020). In addition to photoreceptors, the light-signalling system includes hormones, protein kinases, and transcription factors (TF) (Smith *et al.* 2010, Voitsekhovskaja 2019).

MicroRNAs (miRNAs) also play an important role in light signalling (Gou *et al.* 2011, Samad *et al.* 2017). MicroRNAs are small noncoding RNA molecules that act as posttranscriptional regulators of plant gene expression (Pashkovskiy and Ryazansky 2013). They control a wide range of physiological processes, including mineral nutrition, growth, defence responses, and plant interactions with other organisms both under normal conditions and under stress, by modulating gene expression for transcription factors, stress-induced proteins, enzymes, and hormone metabolism (Voinnet 2009, Sunkar *et al.* 2012, Taylor *et al.* 2014). It is known that the quality of light can affect the transcription of microRNA genes (MIRs). This regulation is achieved through the presence of photosensitive cis-elements in the promoters of these genes. In turn, microRNAs can regulate the processes associated with the response of plants to the light of different intensities and different spectral compositions. For example, microRNAs have been shown to control auxin regulation factors (ARFs) when *A. thaliana* plants are exposed to the blue region of the visible light spectrum (Pashkovskiy *et al.* 2016).

MicroRNAs can be involved in the phytochrome signalling system, particularly through TF (Gou *et al.* 2011, Samad *et al.* 2017). This is consistent with the discovery of promoter regions in miRNA genes that bind TF PIFs, which play an important role in phytochrome signal transduction (Sun *et al.* 2018). It is also known that proteins involved in microRNA processing, DCL1 and HYL1, interact with light-dependent TFs of the bHLH (basic helix-loop-helix) family (to which PIF4 belongs), and destabilize DCL1 during plant irradiation with red light (Sun *et al.* 2018).

A comparison of microRNA expression profiles between *Oryza sativa* wild-type (WT) and *phyb* mutant plants showed that 70 rice genes were targets for 32 differentially expressed miRNAs in mutants (Sun *et al.* 2015). Most of them affect transcription factors, indicating that the regulation of gene expression by miRNAs (such as miR156, miR166, miR171, and miR408) may play an important role in PHY-mediated light signalling. Moreover, it has been shown that the transcription factor HY5, the main regulator of photomorphogenesis, which can interact with several photoreceptors, binds to promoters and regulates the expression of several microRNA genes, such as *MIR156*, *MIR402*, *MIR408*, and *MIR858*) (Zhang *et al.* 2011, Lin *et al.* 2017, Sánchez-Retuerta *et al.* 2018). All the abovementioned factors, together with the effect of PHYB on miRNA levels (Sun *et al.* 2015), indicate that light can regulate the content of mature miRNAs.

In addition to regulating miRNA gene expression, light can also modulate the levels and activity of mature miRNAs. This can be achieved by activating microRNA biogenesis pathways with light. Thus, HYL1 is an RNA-binding protein involved in miRNA processing (Yu *et al.* 2017). In *A. thaliana*, HYL1 protein content is regulated by constitutive photomorphogenic 1 (COP1), an E3 ubiquitin ligase that mediates the proteasomal degradation of light signalling factors (Cho *et al.* 2014, Sánchez-Retuerta *et al.* 2018).

Despite the large amount of data obtained on *A. thaliana* seedlings, there is practically no information on the role of miRNAs in the photomorphogenesis of adult plants. In particular, it is not known what role microRNAs play in photomorphogenesis when plants are grown under BL. To answer this question, in this work, we analysed the expression levels of the main light-dependent miRNAs, most of the associated TFs, as well as some genes for light signalling and microRNA processing in *phya*, *phyb*, and *phyaphyb* mutant *A. thaliana* plants grown under BL, RL, and WL. At the same time, we tried to understand which of the phytochrome receptors plays the main role in the processing of mature miRNAs. It is known that phytochromes can influence photosynthetic processes (Gavassi *et al.* 2017, Kreslavski *et al.* 2018). However, little is known about the relationship between miRNA expression levels, phytochrome content, and photosynthetic processes under light conditions with different spectral compositions. Therefore, we evaluated the effect of light quality and deficiency of phytochromes A and B on photomorphogenesis, photosynthetic processes, and the expression of several light-dependent microRNAs.

Materials and methods

Plant materials and experimental design: Plants of the *Arabidopsis thaliana* WT (Col-0) and mutants (*phyA*, deficient in PHYA CS6219; *phyB*, deficient in PHYB CS71625; *phyaphyb* deficient in PHYA and PHYB CS6224) were used in the experiments. The https://abrc.osu.edu/ – The Ohio State University Arabidopsis Biological Resource Center (USA). The seedlings were germinated for 7 d at 24 ± 1°C, 8-h photoperiod under white fluorescent lamps (58 W/33-640, *Philips*, Poland), at 130 ± 10 μmol(photon) m^{-2} s^{-1} (*LI-COR LI-250A* light meter, USA). Then, the plants were subjected to light with different spectral compositions in individual boxes of the climatic chamber under red (maximum of 660 nm, 24 nm FWHM), blue (maximum of 450 nm, 26 nm FWHM), and white (maxima of 660, 20 nm FWHM and 450 nm, 21 nm FWHM) LEDs (*Epistar*, Taiwan) [130 ± 10 μmol(photon) m^{-2} s^{-1}] for 21 d, 8-h photoperiod. The spectral

characteristics of the light sources were determined using an *AvaSpecULS2048CL-EVO* spectrometer (*Avantes*, The Netherlands) (Fig. 1S, *supplement*). For the fluorescent and photosynthetic measurements, fully developed, healthy-looking upper leaves with almost horizontal leaf blades were used. Each treatment used 6–12 developed upper leaves from three or four plants. All experiments were repeated three or four times (*n*).

Measurements of CO_2 gas exchange: The photosynthetic rate (P_N) was determined in a closed system under light conditions using an *LCPro+* portable infrared gas analyser from *ADC BioScientific Ltd*. (United Kingdom). The CO_2 uptake per leaf area P_N [μmol m^{-2} s^{-1}] was determined. The rate of photosynthesis of the leaves in the second layer from the top was determined at a saturating light intensity of 600 μmol(photon) m^{-2} s^{-1}. The measurements were performed at a light intensity of 600 μmol(photon) m^{-2} s^{-1} as well as before irradiation.

Determination of photochemical activity: Fluorescence parameters characterizing the state of the photosynthetic apparatus were calculated based on induction fluorescence curves obtained using data from the JIP test, which is usually used to evaluate the state of PSII. Chl fluorescence induction curves (OJIP curves) were recorded with the setup described earlier (Kreslavski *et al*. 2014). For the JIP test, OJIP curves were measured under illumination with blue light at an intensity of 6,000 μmol(photon) m^{-2} s^{-1} for 1 s.

Based on induction fluorescence curves (OJIP curves), the following parameters, which characterize PSII photochemical activity, were calculated: F_V/F_M, the PSII maximum quantum photochemical yield, and PI_{ABS}, the PSII performance index (Goltsev *et al.* 2016, Kalaji *et al.* 2016). Here, F_V is the variable fluorescence, which is equal to the difference between F_M and F_0; F_0 is the minimum amplitude of fluorescence (F), and F_M is the maximum amplitude of fluorescence. To calculate PI_{ABS}, the following formula was used: $PI_{ABS} = (F_V/F_M)/(M_0/V_J) \times \times (F_V/F_0) \times (1 - V_J)/V_J$; $M_0 = 4 \times (F_{300\mu s} - F_0)/(F_M - F_0)$; and $V_J = (F_{2ms} - F_0)/(F_M - F_0)$, where M_0 is the average value of the initial slope of the relative variable fluorescence of Chl *a*, which reflects the closing rate of the PSII reaction centres, and V_J is the relative level of fluorescence in phase J after 2 ms.

RNA extraction and RT-PCR: RNA isolation was performed according to the TRIzol reagent method (*Sigma*, Germany). 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. The expression patterns of the genes were assessed using the *CFX96 Touch™* Real-Time PCR Detection System (*Bio-Rad*, USA). Gene-specific primers (Table 1S, *supplement*) for apoproteins of main photoreceptors phytochrome A (*phyA* X17341.1), phytochrome B (*phyB* NM_127435.4), cryptochrome 1 (*Cry1* NM_116961.5), cryptochrome 2 (*Cry2* NM_179257.2), main light signalling transcription factors, phytochrome interacting factor 1 (*PIF1* Q8GZM7), phytochrome interacting factor 3 (*PIF3* NM_001202630.2), phytochrome interacting factor 4 (*PIF4* NM_129862.3), phytochrome interacting factor 5 (*PIF5* Q84LH8), phytochrome interacting factor 7 (*PIF7* NM_001037040.3), transcription factor HY5-like (*HYH* Q8W191), transcription factor HY5 (*HY5* O24646), protein FAR-RED ELONGATED HYPOCOTYL 1 (*FHY1* Q8S4Q6), protein FAR-RED ELONGATED HYPOCOTYL 3 (*FHY3* Q9LIE5), transcription factor HFR1 (*HFR1* Q9FE22), squamosa promoter-binding-like protein 7 (*SPL7* Q8S9G8), endoribonuclease Dicer homologue 1 (*DCL1* Q9SP32), small RNA 2'-O-methyltransferase (*HEN1* Q9C5Q8), double-stranded RNA-binding protein 1 (*HYL1* O04492) were selected using nucleotide sequences from the *National Center for Biotechnology Information* (NCBI) database (https://www.ncbi.nlm.nih.gov/, USA) and https://www.uniprot.org/ with *Vector NTI Suite 9* software (*Invitrogen*, USA). The transcript levels were normalized to the expression of the *Actin1* gene. The experiments were performed with three biological and analytical replicates.

The microRNA extraction was performed with a *mirPremier* microRNA isolation kit (*Sigma*, Germany). The expression patterns of microRNAs were assessed by real-time PCR using a *QuantStudio 1* real-time PCR system (*Thermo Fisher Scientific*, USA). cDNA was synthesized by ligation-mediated reverse transcription using a *miScript Plant RT* Kit (*Qiagen*, The Netherlands) according to the manufacturer's protocol. qRT-PCR reactions were performed according to the manufacturer's instructions (*miScript SYBR Green PCR* Kit, *Qiagen*, The Netherlands) using *miScript* universal primers and miRNA-specific primers (Table 1S) and cDNA templates. Gene-specific primers were selected using nucleotide sequences from the https://www.mirbase.org/ database (United Kingdom) with *Vector NTI Suite 9* software (*Invitrogen*, USA). The gene expression patterns of the microRNA expression levels were normalized to the expression of small nucleolar RNA U6.

Statistical data processing: The experiments were performed in three biological replicates and three analytical replicates. The expression level of each gene was measured in three independent experiments. For each of these experiments, at least three parallel independent measurements were performed. The significance of the differences between the groups was calculated by one-way analysis of variance (*ANOVA*) followed by *Duncan*'s method using *SigmaPlot 12.3* (*Systat Software Inc*., USA). Asterisks indicate significant differences between WT and mutants (*$p<0.05$; **$p<0.01$). The mRNA and microRNA levels of the genes were expressed as the fold change $\log_2$. The data are shown as the mean ± SD ($n = 3$). The presented values are from at least three biological replications. For the fluorescence and CO_2 gas-exchange measurements, six to twelve fully developed leaves from three or four plants were used.

Results

Morphological features: Growing wild-type plants under BL caused earlier flowering and the appearance of larger leaves, as well as an increase in their area (more than 3,000 mm^2), and under RL, the formation of longer petioles and thick drooping leaves and a decrease in the leaf area by almost 3 times (Fig. 1, Table 1). The *phya* mutant under RL had a leaf area that decreased to 286 ± 58 mm^2, while under BL, the usual early-flowering phenotype (leaf area of 790 ± 64 mm^2) appeared. The *phyb* mutant reacted negatively to the presence of a large proportion of RL in the spectrum. Therefore, under WL, in the spectrum of which RL dominated, long petioles with small round leaves (leaf area of 60 mm^2) were formed; cultivation under RL caused folding of the leaf blade and its thinning, strong elongation of leaf petioles, and early flowering (leaf area of 381 ± 61 mm^2), while phenotype normalization was observed under BL (leaf area of 907 ± 99 mm^2). The *phyaphyb* mutant showed dwarfism more than the *phyb* mutant. The presence of RL in the spectrum (leaf area of 25 mm^2 WL and RL) under BL slightly improved the phenotype, and *phyaphyb* plants (leaf area of 320 ± 76 mm^2) flowered even faster than WT plants (Fig. 1).

Fluorescent parameters: The effective and maximum quantum yields of PSII did not significantly differ in mutants and WT in almost all experimental variants (Table 1), except for the *phyb* mutant grown under RL, whose $Y_{(II)}$ value was 0.37 ± 0.2, while in WT, this index was 0.46 ± 0.03. In the *phyb* and *phyaphyb* mutants grown under BL, the $Y_{(II)}$ values were higher than those in the same mutants grown under RL or WL. The PSII performance indicator PI_{ABS} did not significantly differ between *phya* and WT in all variants, while when plants were grown under BL, this parameter was higher in the *phyb* mutant than that in the WT and *phya* mutants. At the same time, the PI_{ABS} value was lower in the *phyb* and *phyaphyb* mutants than that in the WT and *phya* plants grown under RL and WL. In the *phyb* and *phyaphyb* mutants grown under BL, the PI_{ABS} value was 2–4 times higher than that in the same mutants grown under RL and WL. The nonphotochemical quenching index, NPQ, was more than 1.5 times higher in WT compared to mutant plants grown under RL and WL but not under BL, where this indicator did not significantly differ from other variants (Table 1).

Photosynthetic rate: The P_N value in WT plants grown under WL was higher than that in all studied mutants. In RL plants, the highest P_N was in the WT and *phya* mutant, and the lowest was in *phyaphyb*. In BL plants, WT had the lowest rate, while the rates of photosynthesis in other variants were comparable. At the same time, the P_N in mutants grown under RL and BL was approximately two times higher than that in the same mutants grown under WL (Table 1).

Gene expression: Functionally, the studied genes can be divided into several groups: genes for photoreceptor apoproteins, genes for light-signalling apoproteins,

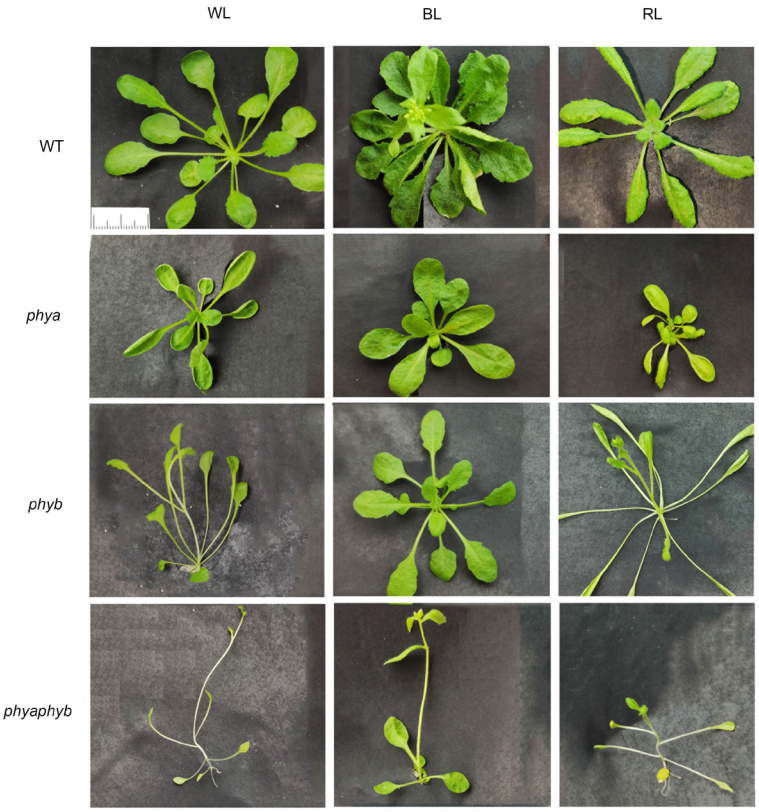

Fig. 1. Effect of light with different spectral composition on the phenotype of *Arabidopsis thaliana* phytochrome mutants. BL – blue light; RL – red light; WL – white light.

Table 1. Effect of light with different spectral composition on the Chl *a* fluorescence parameters, the photosynthetic rates (P_N), and leaf area in *Arabidopsis thaliana* phytochrome mutants. *Different letters* indicate significant differences between samples ($p<0.05$). The means ± standard errors are presented, $n = 3$. BL – blue light; RL – red light; WL – white light. $Y_{(II)}$ – PSII effective quantum yield; ETR – relative electron transport rate; q_P – coefficient of photochemical fluorescence quenching; $Y_{(NO)}$ – quantum yield of nonregulated nonphotochemical energy dissipation in PSII; $Y_{(NPQ)}$ – quantum yield of regulated nonphotochemical energy dissipation in PSII; q_N – coefficient of nonphotochemical fluorescence quenching; q_L – coefficient of photochemical fluorescence quenching assuming interconnected PSII light-harvesting antennae; NPQ – nonphotochemical fluorescence quenching; F_V/F_M – PSII maximum quantum yield; PI_{ABS} – PSII performance index; P_N – net photosynthetic rate.

	WT	*phya*	*phyb*	*phyaphyb*
WL				
$Y_{(II)}$	0.41 ± 0.01[a]	0.42 ± 0.03[a]	0.38 ± 0.02[a]	0.39 ± 0.04[a]
ETR	32.65 ± 0.64	33.78 ± 2.16	29.85 ± 3.96	33.50 ± 2.54
q_P	0.66 ± 0.00	0.63 ± 0.04	0.57 ± 0.05	0.58 ± 0.03
q_N	0.66 ± 0.02	0.53 ± 0.03	0.56 ± 0.04	0.47 ± 0.05
q_L	0.43 ± 0.01	0.36 ± 0.04	0.31 ± 0.03	0.31 ± 0.02
NPQ	1.28 ± 0.10[a]	0.81 ± 0.08[b]	0.92 ± 0.09[b]	0.89 ± 0.08[b]
$Y_{(NO)}$	0.26 ± 0.02	0.32 ± 0.03	0.32 ± 0.10	0.31 ± 0.10
$Y_{(NPQ)}$	0.33 ± 0.01	0.26 ± 0.00	0.30 ± 0.05	0.20 ± 0.05
F_V/F_M	0.79 ± 0.01[a]	0.78 ± 0.01[a]	0.78 ± 0.01[a]	0.77 ± 0.01[a]
PI_{ABS}	2.1 ± 0.3[a]	2.1 ± 0.2[a]	1.1 ± 0.3[b]	1.2 ± 0.2[b]
P_N [μmol(CO_2) m^{-2} s^{-1}]	6.6 ± 0.5[a]	4.1 ± 0.2[b]	2.9 ± 0.2[c]	3.3 ± 0.2[c]
Leaf area [mm^2]	1,598 ± 228[a]	538 ± 84[b]	63 ± 25[c]	25 ± 12[c]
BL				
$Y_{(II)}$	0.51 ± 0.02[a]	0.45 ± 0.02[a]	0.46 ± 0.03[a]	0.53 ± 0.03[a]
ETR	40.80 ± 1.13	35.84 ± 1.06	37.01 ± 0.79	42.42 ± 0.87
q_P	0.74 ± 0.02	0.65 ± 0.01	0.64 ± 0.01	0.75 ± 0.02
q_N	0.53 ± 0.04	0.50 ± 0.04	0.47 ± 0.02	0.52 ± 0.01
q_L	0.46 ± 0.02	0.37 ± 0.01	0.33 ± 0.01	0.47 ± 0.04
NPQ	0.85 ± 0.07[a]	0.75 ± 0.05[a]	0.67 ± 0.04[a]	0.80 ± 0.04[a]
$Y_{(NO)}$	0.26 ± 0.01	0.31 ± 0.03	0.32 ± 0.00	0.26 ± 0.01
$Y_{(NPQ)}$	0.22 ± 0.02	0.24 ± 0.02	0.22 ± 0.01	0.21 ± 0.01
F_V/F_M	0.81 ± 0.01[a]	0.80 ± 0.01[a]	0.81 ± 0.01[a]	0.81 ± 0.00[a]
PI_{ABS}	3.8 ± 0.5[a]	3.8 ± 0.3[a]	4.8 ± 0.2[a]	4.5 ± 0.5[a]
P_N [μmol(CO_2) m^{-2} s^{-1}]	4.8 ± 0.4[b]	8.2 ± 0.2[a]	10.0 ± 1.1[a]	8.5 ± 0.5[a]
Leaf area [mm^2]	3,164 ± 321[a]	790 ± 64[b]	907 ± 99[b]	320 ± 76[c]
RL				
$Y_{(II)}$	0.46 ± 0.03[a]	0.45 ± 0.05[a]	0.37 ± 0.02[b]	0.40 ± 0.04[ab]
ETR	36.57 ± 0.19	36.07 ± 4.20	29.35 ± 0.56	35.56 ± 3.55
q_P	0.66 ± 0.00	0.63 ± 0.04	0.57 ± 0.01	0.62 ± 0.05
q_N	0.52 ± 0.00	0.46 ± 0.09	0.53 ± 0.01	0.49 ± 0.04
q_L	0.37 ± 0.00	0.32 ± 0.00	0.31 ± 0.01	0.32 ± 0.04
NPQ	0.81 ± 0.04[a]	0.67 ± 0.10[a]	0.71 ± 0.04[a]	0.73 ± 0.09[a]
$Y_{(NO)}$	0.30 ± 0.00	0.33 ± 0.01	0.35 ± 0.01	0.32 ± 0.01
$Y_{(NPQ)}$	0.24 ± 0.00	0.22 ± 0.06	0.28 ± 0.01	0.23 ± 0.03
F_V/F_M	0.81 ± 0.01[a]	0.81 ± 0.01[a]	0.77 ± 0.01[b]	0.78 ± 0.01[ab]
PI_{ABS}	2.9 ± 0.3[a]	2.9 ± 0.3[a]	1.8 ± 0.1[b]	2.0 ± 0.2[b]
P_N [μmol(CO_2) m^{-2} s^{-1}]	8.0 ± 0.4[a]	7.3 ± 0.2[a]	6.9 ± 0.8[a]	6.1 ± 0.4[b]
Leaf area [mm^2]	1,222 ± 105[a]	286 ± 58[b]	381 ± 61[b]	23 ± 12[c]

including transcription factors, and genes for microRNA processing. We considered significant changes in expression if the level of transcripts changed by at least two times (log_2).

Genes of photoreceptor apoproteins, light signalling, and microRNA processing: In WT, predictable light-dependent expression of the apoprotein genes of the main studied photoreceptors was observed. For example, there

was an increase in the levels of transcripts of the *PHYA* and *PHYB* apoprotein genes in the RL variant and the *PHYA* apoprotein under WL, where a large amount of RL is also present (Fig. 2*B*,*C*). At the same time, the expression of phytochromes in BL plants did not noticeably change. In the *phya* mutant under RL and BL, a decrease in the transcripts of the *PHYB* and *PHYA* apoproteins was observed. In the *phyaphyb* mutant, a marked decrease in the expression of photoreceptor apoproteins was observed in all light variants (Fig. 2*B*–*D*).

In WT, the levels of transcripts of the *PIF1* and *PIF5* genes increased by more than 2 times under BL, while the level of *PIF3* expression decreased under the same conditions. In RL plants, the expression of the *PIF4* and *PIF5* genes increased by more than 2 times (Fig. 2*A*). The transcript level of *HY5* decreased in all variants in WT by more than two times (Fig. 2*A*). In the *phya* mutant, the expression of the *PIF1* gene significantly decreased under BL and WL, and *PIF7* significantly decreased under WL (Fig. 2*B*). The level of *HY5* gene transcripts in the *phya* mutant also significantly decreased in all light variants, as in WT, while an increase in the level of *HYH* transcripts in the *phya* mutant under BL was observed (more than 2 times) (Fig. 2*B*). In the *phyb* mutant, the levels of transcripts of the *PIF*s, *HYH*, and *HY5* genes decreased or changed little in all variants of the experiment, except for the WL variant, in which the expression of the *HY5* gene increased by more than 4 times. In the *phyaphyb* mutant, the expression of the *PIF1* gene increased 2-fold in RL and BL, while in the *PIF4* gene, it increased only in BL.

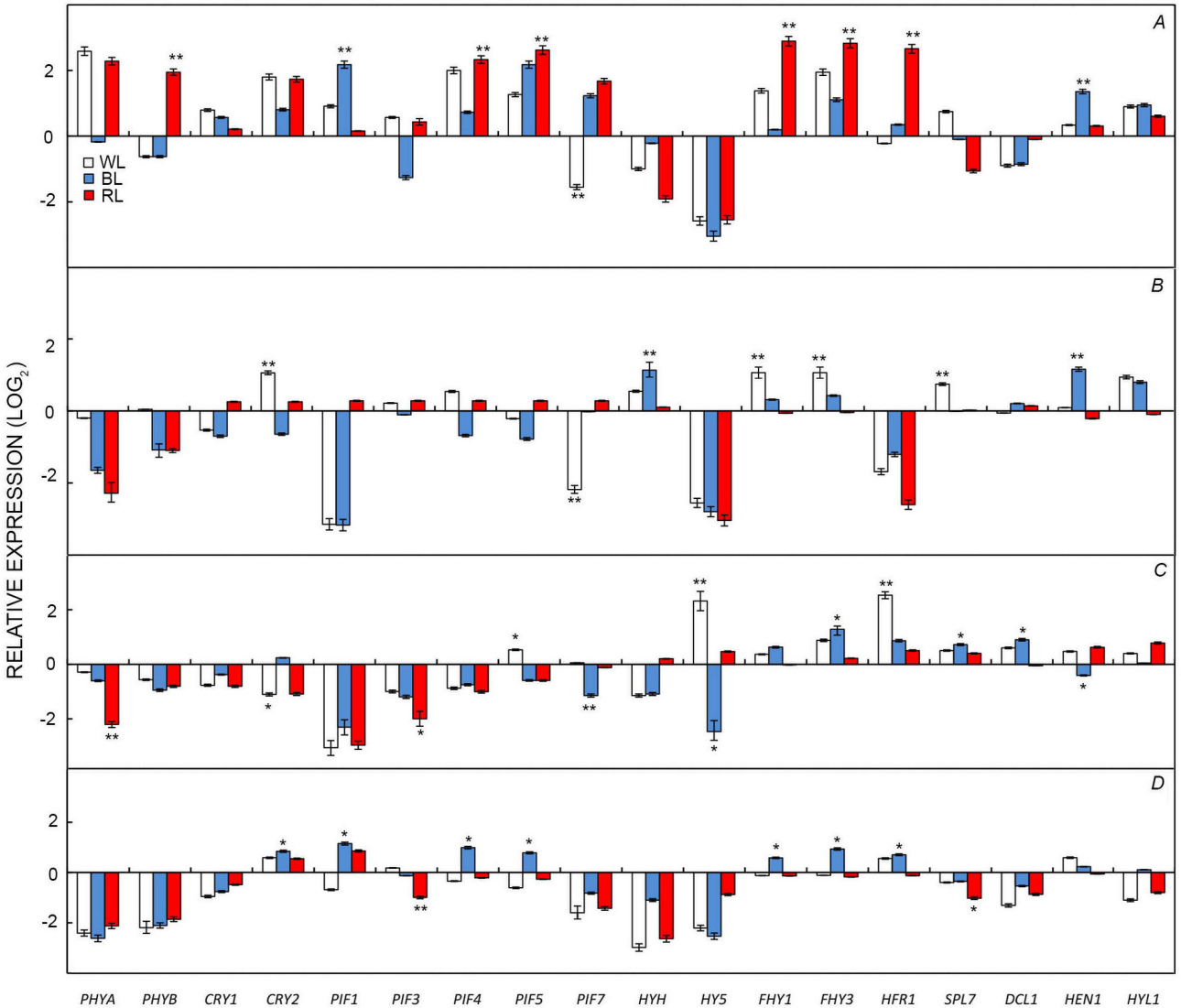

Fig. 2. Effect of light with different spectral composition on the expression of transcription factor genes, light-signalling genes, and processing microRNA. (*A*) Wild type, (*B*) *phya* mutant, (*C*) *phyb* mutant, and (*D*) *phyaphyb* mutant. Log_2 data are presented. Changes were considered significant if the expression was above or below one. *Asterisks* indicate significant differences between samples (*p<0.05; **p<0.01). The means ± standard errors are presented, n = 3. BL – blue light; RL – red light; WL – white light.

The expression of all other TFs decreased or changed little in all variants of the experiment (Fig. 2*C*,*D*).

In WT, all studied light-signalling genes generally tended to increase. The greatest increase in expression was observed in the *FHY1*, *FHY3*, and *HFR1* genes when exposed to RL (an increase of 2–4 times). In the *phya* mutant, only WL caused an increase in the *FHY1* and *FHY3* transcript levels by 2 times, while the expression of the *HFR1* gene decreased in all studied variants (Fig. 2*B*). In the *phyb* mutant, WL caused an increase in the transcript level of the *HFR1* gene by more than 2 times, and in the *FHY3* gene, expression under WL and BL increased by 2 times. In the *phyaphyb* mutant, the level of *FHY3* gene transcripts increased 2-fold under BL, and the expression levels of other genes changed insignificantly (Fig. 2*D*).

In WT, in the BL variant, the expression of the *HEN1* gene increased by 2 times (Fig. 2*A*), while the expression of the *DCL1* gene decreased by 2 times under WL and BL, and the level of *SPL7* gene transcripts decreased by 2 times in the RL variant. In the *phya* mutant, the expression of the *HEN1* gene also increased 2-fold under BL; at the same time, the expression of the *HYL1* gene under WL and BL increased almost 2-fold. In the *phyb* mutant, there was an increase in the transcript level of the *DCL1* gene under BL by almost 2 times. In the double mutant, the expression of all microRNA processing genes decreased, but the strongest drop in transcript levels (by a factor of 2 or more) was observed in the *DCL1* and *HYL1* genes under WL and in *SLP7* under RL. Of note, the expression of the *HYL1* and *DCL1* genes in plants grown under BL was higher than that in the corresponding plant genes under RL and WL (Fig. 2).

MicroRNA expression: The expression of most genes of the studied miRNAs was increased in WT when grown under BL and, in particular, under WL (Fig. 3*A*). Thus, in BL plants, the expression of all studied miRs increased by more than 2 times, except for miR319c, miR163-5p, miR827, and miR398. At the same time, under RL conditions, the expression of miR396a-3p, miR168a-5p, miR170/171-5p, miR858a, miR156/157-5p, and miR408 increased by more than 4 times. Of note, miR396a-3p, miR402, miR168a-5p, miR833a-5p, miR858, and miR156/157-5p under WL were expressed at a high level in WT (Fig. 3*A*). In the *phya* mutant, the expression of miR319c, miR163-5p, and miR166a-5p increased by more than 2–4 times under WL. At the same time, miR402, miR168a-5p, miR827, and miR472 expression decreased by more than 2-fold under the same conditions. In the *phya* mutant under BL, the expression of miR319c, miR163-5p, miR402, miR166a-5p, and miR397a increased by 2–4 times. In the WL variant, the expression of miR319c and miR163-5p increased by more than 2 times in the *phya* mutant, while miR165a-5p, miR166a-5p, and miR168a-5p, miR172a, miR827, and miR472-5p significantly decreased. In the RL variant in the *phya* mutant, the expression of miR160, miR319c, miR172a, miR833a-5p, miR827, miR170/171-5p, and miR156/157-5p decreased by 2 or more times (Fig. 3*B*). At the same time, the expression levels of miR163-5p, miR166a-5p, miR397a, miR398, and miR408 increased 2-fold in the *phya* mutant in the RL variant (Fig. 3*B*). In *the phyb* mutant, the main difference was an increase in the expression of almost all studied miRs under the action of red and especially BL by 2–4 times, except for miR397a, miR319c, and miR408, the expression of which decreased. Under white light, miR expression did not change, except for miR833a-5p, miR827, and miR397a, miR319c, and miR408 expression, which decreased. In the *phyaphyb* double mutant, the expression of all miRs increased by more than 2 times under blue light, except for one miR398c, while WL and RL caused a decrease in the intensity of expression of all studied miRs (Fig. 3*C*,*D*).

Discussion

The quality of light influenced the photomorphogenesis of plants, among which the *phyb* and *phyaphyb* mutants had significant differences from WT and *phya* mutants; the predominance of RL in the emission spectrum of light sources led to the reduction of leaf blades and elongation of leaf petioles in the mutants (Fig. 1). Under the BL, in *phyb* and *phyaphyb* mutants, the effect found under RL was not manifested because all phytochromes and light-signalling elements were less active under BL conditions (Fig. 2*B*,*D*). MiRNAs miR160, miR167, and miR848 affect the elongation of *Arabidopsis* hypocotyls under RL (Sun *et al.* 2018). In our experiments in adult plants, the expression of miR160 and miR167 increased by RL in the *phyb* mutant and WT, while a decrease was observed in the *phya* mutant, and the highest values of the abovementioned miRNAs were in the *phyb* and *phyaphyb* mutants in the BL variant (Fig. 3*B–D*).

A noticeable decrease in PSII activity and intensity of photosynthesis in RL and WL plants was observed only under PHYB deficiency. It is assumed that PHYB, in contrast to PHYA, is more important for photosynthetic apparatus (PA) adaptation in WL and RL plants (Table 1). However, in BL plants, PHYB is not involved in PA adaptation due to weak PHY absorbance in this region, resulting in a low level of the active form of PHY. This is consistent with the fact that *phyb* and *phyaphyb* WL and RL plants had the lowest rates of photosynthesis, and under BL conditions, the rates exceeded those of WT (Table 1). The photosynthetic rates and activity of PSII evaluated by the value of PI_{ABS} in *phya* mutant and WT grown on WL were significantly higher than the photosynthetic rates of *phyb* and *phyaphyb* mutants and respectively, leaf areas were noticeably bigger. Also, the same correlation between the values of photosynthetic rates and leaf area was observed for WT and *phyaphyb* mutant grown on RL. At the same time, it was surprising for us that under BL the photosynthetic rate in *phyb* mutant was higher than that in WT. It can be assumed that PHYB deficiency under BL conditions does not significantly affect the activity of photosynthetic processes.

The response of plants to light quality involves photoreceptors, as well as transcription factors and other signalling molecules, the relative content of which, in certain cases, can be estimated from the level of the

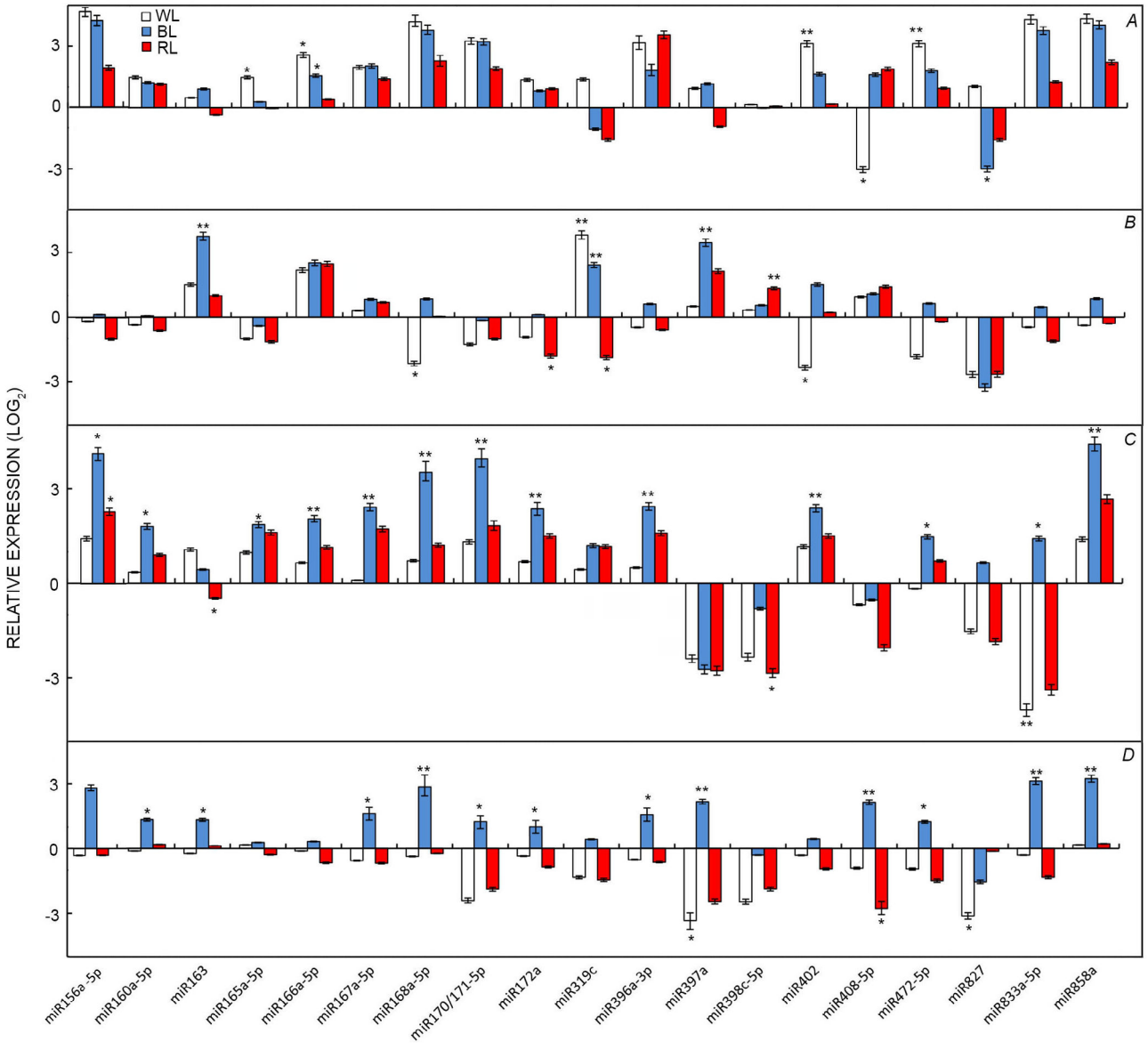

Fig. 3. Effect of light with different spectral composition on the expression of light-dependent miRNAs. (*A*) Wild type, (*B*) *phya* mutant, (*C*) *phyb* mutant, (*D*) *phyaphyb* mutant. Log_2 data are presented. Changes are considered significant if expression is above or below one. *Asterisks* indicate significant differences between samples (*p<0.05; **p<0.01). The means ± standard errors are presented, n = 3. BL – blue light; RL – red light; WL – white light.

corresponding transcripts (Liao *et al.* 2020). The main difference between *phyaphyb* mutant and WT in terms of photoreceptor apoprotein gene transcript levels was a reduced response to the quality of the light used in the experiments (Fig. 2*D*). An important observation was that in *phyb*, the expression of the TFs *HY5* and *HFR1* genes increased by more than 4 times under WL conditions, which was not observed in other mutants and WT. HY5 interacts with the TFs far-red elongated hypocotyl 1 (HFR1) and long after far-red light 1 (LAF1), preventing their degradation (Jang *et al.* 2013). At the same time, the interaction of HY5 with the TFs such as far-red elongated hypocotyl 3 (FHY3) and far-red impaired response 1 (FAR1) prevents their functioning (Lin *et al.* 2007). In turn, TF FHY3 and FAR1 are required for the expression of FHY1 (Li *et al.* 2010), which regulates phyA transport into the nucleus (Hiltbrunner *et al.* 2005). Downregulation of FHY3 and FAR1 by HY5 is important for PHYA-mediated signalling in seedlings and, in our opinion, PHYB-mediated signalling in adult *A. thaliana* plants (Fig. 2). Moreover, HY5 binds to promoters and regulates the expression of several *A. thaliana* miRNA genes, such as *MIR156*, *MIR402*, *MIR408*, and *MIR858* (Zhang *et al.* 2011). In our experiments, the highest miRNA expression was observed in *phyb* mutants, which was accompanied by a decrease in the expression of *HY5*, *PIF4*, and *PIF5*

genes (Fig. 2*C*). In addition, the transcription of *HY5* itself is regulated by several microRNAs such as miR157 and miR319. In turn, miR157 and miR319 are dependent on the 2'-O-methyltransferase HEN1. In deetiolated seedlings, HEN1 accumulation is accompanied by an increase in the levels of mature miR157 and miR319 (Tsai *et al.* 2014). HY5 can activate *HEN1* expression by forming a negative regulatory connection that is mediated by miR157 because this miRNA ultimately targets the inhibition of HY5 transcripts (Tsai *et al.* 2014, Hernando *et al.* 2017, Sánchez-Retuerta *et al.* 2018). In our experiments, the level of *HEN1* transcripts increased by more than 2 times only in WT and *phya*, while the level of *HY5* expression was reduced in all variants except for the WL *phyb* mutant (more than a 4-fold increase), which was consistent with increased expression of miR157 under BL and decreased expression under WL (Fig. 2*C*). In our experiments, the expression of miR319 decreased in the WT and *phyaphyb* mutant under RL and BL, while in the *phya* and *phyb* mutants in the BL variant, an increase in miR319 expression by more than 2 times was observed (Fig. 3).

Other important transcription factors involved in light signalling and negatively regulated are PIFs. At the same time, some PIFs can regulate the expression of light-dependent miRNAs. Thus, *MIR156* is a PIF5 target gene (Hornitschek *et al.* 2012, Xie *et al.* 2017). It represses transcription by directly binding to cis-elements in their promoters. This causes a decrease in the levels of mature miR156 and a concomitant increase in the number of SPL TF transcripts that are targets of miR156. In mutants with damaged photoreceptors, a violation of the PHYB–Pfr–PIF interaction is assumed, which should cause a decrease in the regulatory ability of TFs. We observed a decrease in the expression of most PIFs studied in mutants, with the greatest decrease occurring in *phyb* in all variants, although there were no significant changes in the level of *SPL* transcripts (Figs. 2, 3).

Under BL conditions, we observed an increase in the expression of the studied light-dependent microRNAs associated with ontogenetic and morphological development (miR160, miR165, miR163, miR402, miR168, miR172, miR170, miR166, miR167, and miR156) (Fig. 3), which was accompanied by normalization of the phenotype under BL in *phyb* and *phyaphyb* mutants and, as a result, an increase in CO_2 gas exchange (Fig. 3*C*,*D*; Table 1). We suggest that the phenotypic response of plants under WL and RL conditions is due to reduced expression of microRNAs (miR319, miR172, miR833, miR472) in the *phyaphyb* mutant (Fig. 3*D*). PIFs can also influence miRNA processing proteins. HYL1 (dsRNA-binding domain-like superfamily protein, HYPONASTIC LEAVES 1) and DCL1 (endoribonuclease Dicer-1) are key regulators of microRNA biogenesis. HYL1 protein levels are also controlled by PIFs (Sun *et al.* 2018). Both HYL1 and DCL1 interact with PIFs, which inhibit them under RL conditions and activate them in the dark. *Dcl1* and *hyl1* mutants show shorter hypocotyls than wild-type plants under RL, indicating that DCL1 and HYL1 play a negative role in photomorphogenesis (Sun *et al.* 2018). In our experiments, the level of *HYL1* transcripts increased in WT and *phya* mutants by more than 2 times under WL and BL; in addition, in the *phyaphyb* mutant, RL and WL caused a decrease in expression by more than 2 times (Fig. 2*B*,*D*). It is important to note the increase in DCL1 expression in *phyb* under BL, which is also consistent with the high expression intensity of most light-dependent miRs in this variant.

Conclusion: Conserved light-dependent miRNAs are involved in adult plant photomorphogenesis not only under RL but also under BL. In these processes, the most important role is played by the phytochrome system in general and by PHYB in particular, as evidenced by an increase in the expression of most light-dependent microRNA genes and the increase in microRNA processing under BL conditions. In the studied mutants under BL, the activity of phytochromes is reduced, which can lead to a decrease in the negative regulation of PIFs and, as a result, to an increase in the level of expression of light-dependent microRNAs (which, in our opinion, may be related to the normalization of the phenotype) as well as an increase in the intensity of photosynthesis. It can be assumed that it is not the activation of the blue light photoreceptor system but more complete inactivation of the phytochrome system under BL, primarily PHYB, that can positively regulate the processing of mature miRNAs.

References

Chaves I., Pokorny R., Byrdin M. *et al.*: The cryptochromes: blue light photoreceptors in plants and animals. – Annu. Rev. Plant Biol. **62**: 335-364, 2011.

Cho S.K., Chaabane S.B., Shah P. *et al.*: COP1 E3 ligase protects HYL1 to retain microRNA biogenesis. – Nat. Commun. **5**: 5867, 2014.

Gavassi M.A., Monteiro C.C., Campos M.L. *et al.*: Phytochromes are key regulators of abiotic stress responses in tomato. – Sci. Hortic.-Amsterdam **222**: 126-135, 2017.

Goltsev V.N., Kalaji H.M., Paunov M. *et al.*: Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. – Russ. J. Plant Physiol. **63**: 869-893, 2016.

Gou J., Felippes F.F., Liu C. *et al.*: Negative regulation of anthocyanin biosynthesis in *Arabidopsis* by a miR156-targeted SPL transcription factor. – Plant Cell **23**: 1512-1522, 2011.

Hayes S., Velanis C.N., Jenkins G.I., Franklin K.A.: UV-B detected by the UVR8 photoreceptor antagonizes auxin signaling and plant shade avoidance. – P. Natl. Acad. Sci. USA **111**: 11894-11899, 2014.

Hernando C.E., Garcia C., Mateos J.L.: Casting away the shadows: elucidating the role of light-mediated posttranscriptional control in plants. – Photochem. Photobiol. **93**: 656-665, 2017.

Hiltbrunner A., Viczián A., Bury E. *et al.*: Nuclear accumulations of the phytochrome A photoreceptor requires FHY1. – Curr. Biol. **15**: 2125-2130, 2005.

Hornitschek P., Kohnen M.V., Lorrain S. *et al.*: Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. – Plant J. **71**: 699-711, 2012.

Jang I.-C., Henriques R., Chua N.-H.: Three transcription factors, HFR1, LAF1 and HY5, regulate largely independent

signaling pathways downstream of phytochrome A. – Plant Cell Physiol. **54**: 907-916, 2013.
Kalaji H.M., Jajoo A., Oukarroum A. *et al.*: Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. – Acta Physiol. Plant. **38**: 102, 2016.
Kong S.-G., Okajima K.: Diverse photoreceptors and light responses in plants. – J. Plant Res. **129**: 111-114, 2016.
Kreslavski V.D., Lankin A.V., Vasilyeva G.K. *et al.*: Effects of polyaromatic hydrocarbons on photosystem II activity in pea leaves. – Plant Physiol. Bioch. **81**: 135-142, 2014.
Kreslavski V.D., Los D.A., Schmitt F.-J. *et al.*: The impact of the phytochromes on photosynthetic processes. – BBA-Bioenergetics **1859**: 400-408, 2018.
Kreslavski V.D., Strokina V.V., Pashkovskiy P.P. *et al.*: Deficiencies in phytochromes A and B and cryptochrome 1 affect the resistance of the photosynthetic apparatus to high-intensity light in *Solanum lycopersicum*. – J. Photoch. Photobio. B **210**: 111976, 2020.
Li J., Li G., Gao S. *et al.*: *Arabidopsis* transcription factor ELONGATED HYPOCOTYL5 plays a role in the feedback regulation of phytochrome A signaling. – Plant Cell **22**: 3634-3649, 2010.
Liao X., Liu W., Yang H., Jenkins G.I.: A dynamic model of UVR8 photoreceptor signalling in UV-B acclimated *Arabidopsis*. – New Phytol **227**: 857-866, 2020.
Lin M.-C., Tsai H.-L., Lim S.-L. *et al.*: Unraveling multifaceted contributions of small regulatory RNAs to photomorphogenic development in *Arabidopsis*. – BMC Genomics **18**: 559, 2017.
Lin R., Ding L., Casola C. *et al.*: Transposase-derived transcription factors regulate light signaling in *Arabidopsis*. – Science **318**: 1302-1305, 2007.
Möglich A., Yang X., Ayers R.A., Moffat K.: Structure and function of plant photoreceptors. – Annu. Rev. Plant Biol. **61**: 21-47, 2010.
Pashkovskiy P.P., Kartashov A.V., Zlobin I.E. *et al.*: Blue light alters miR167 expression and microRNA-targeted auxin response factor genes in *Arabidopsis thaliana* plants. – Plant Physiol. Bioch. **104**: 146-154, 2016.
P.P., Ryazansky S.S.: Biogenesis, evolution, and functions of plant microRNAs. – Biochemistry-Moscow **78**: 627-637, 2013.
Quail P.H.: Phytochromes. – Curr. Biol. **20**: R504-R507, 2010.
Samad A.F.A., Sajad M., Nazaruddin N. *et al.*: MicroRNA and transcription factor: key players in plant regulatory network. – Front. Plant Sci. **8**: 565, 2017.
Sánchez-Retuerta C., Suaréz-López P., Henriques R.: Under a new light: regulation of light-dependent pathways by non-coding RNAs. – Front. Plant Sci. **9**: 962, 2018.
Smith K.M., Sancar G., Dekhang R. *et al.*: Transcription factors in light and circadian clock signaling networks revealed by genomewide mapping of direct targets for *Neurospora* white collar complex. – Eukaryotic Cell **9**: 1549-1556, 2010.
Su J., Liu B., Liao J. *et al.*: Coordination of cryptochrome and phytochrome signals in the regulation of plant light responses. – Agronomy **7**: 25, 2017.
Sullivan J.A., Deng X.W.: From seed to seed: the role of photoreceptors in *Arabidopsis* development. – Dev. Biol. **260**: 289-297, 2003.
Sun W., Xu X.H., Wu X. *et al.*: Genome-wide identification of microRNAs and their targets in wild type and *phyb* mutant provides a key link between microRNAs and the *phyb*-mediated light signaling pathway in rice. – Front. Plant Sci. **6**: 372, 2015.
Sun Z., Li M., Zhou Y. *et al.*: Coordinated regulation of *Arabidopsis* microRNA biogenesis and red light signaling through Dicer-like 1 and phytochrome-interacting factor 4. – PLoS Genet. **14**: e1007247, 2018.
Sunkar R., Li Y.-F., Jagadeeswaran G.: Functions of microRNAs in plant stress responses. – Trends Plant Sci. **17**: 196-203, 2012.
Taylor R.S., Tarver J.E., Hiscock S.J., Donoghue P.C.J.: Evolutionary history of plant microRNAs. – Trends Plant Sci. **19**: 175-182, 2014.
Tsai H.-L., Li Y.-H., Hsieh W.-P. *et al.*: HUA ENHANCER1 is involved in posttranscriptional regulation of positive and negative regulators in *Arabidopsis* photomorphogenesis. – Plant Cell **26**: 2858-2872, 2014.
Voinnet O.: Origin, biogenesis, and activity of plant microRNAs. – Cell **136**: 669-687, 2009.
Voitsekhovskaja O.V.: Phytochromes and other (photo)receptors of information in plants. – Russ. J. Plant Physiol. **66**: 351-364, 2019.
Xie Y., Liu Y., Wang H. *et al.*: Phytochrome-interacting factors directly suppress *MIR156* expression to enhance shade-avoidance syndrome in *Arabidopsis*. – Nat. Commun. **8**: 348, 2017.
Yu Y., Jia T., Chen X.: The 'how' and 'where' of plant microRNAs. – New Phytol. **216**: 1002-1017, 2017.
Zhang H., He H., Wang X. *et al.*: Genome-wide mapping of the *HY5*-mediated gene networks in *Arabidopsis* that involve both transcriptional and post-transcriptional regulation. – Plant J. **65**: 346-358, 2011.

© The authors. This is an open access article distributed under the terms of the Creative Commons BY-NC-ND Licence.