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Role of anthocyanin and carotenoids in the adaptation of the photosynthetic apparatus of purple- and green-leaved cultivars of sweet basil (*Ocimum basilicum*) to high-intensity light

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Abstract

Comparative studies of the adaptation of green- and purple-leaved *Ocimum basilicum* varieties, which differ in carotenoid and anthocyanin contents, to high-intensity light have been carried out. The plants were grown for 21 d under white light-emitting diode providing different light intensities [300, 550, and 1,350 μmol(photon) m^{-2} s^{-1}]. During the experiment, fresh mass and relative water content, photosynthetic pigment and anthocyanin content, amount of total free phenolic compounds, and the contents of thiobarbituric acid reactive substances and H_2O_2 were analysed. The fluorescent parameters, which characterized the PSII activity in the leaves (F_v/F_m, $Y_{(II)}$), as well as the efficiency of dissipation of absorbed energy into heat ($Y_{(NPQ)}$,$Y_{(NO)}$) and the fraction of the PSII open centres (q_L), were determined. The obtained results are consistent with the concept of the important role of not only anthocyanin but also carotenoids in plant adaptation to high-intensity light.

Additional key words: chlorophyll *a* fluorescence; long-term growth; narrow-band diodes.

Introduction

High-intensity light (HIL) is a major stress factor that negatively affects the function of the photosynthetic apparatus (PA). During daylight, most plants are subjected to varying degrees of HIL exposure. Among the components of the PA, PSII and the CO_2 fixation system are the most sensitive to light excess (Aro *et al.* 1993, Andersson and Aro 2001, Huang *et al.* 2018). Under conditions of light stress, when excessively absorbed light energy cannot be used in photochemical reactions, photoinhibition occurs, accompanied by a decrease in the intensity of photosynthesis, formation of reactive oxygen species (ROS), photooxidation of pigments, and destruction of the chloroplast structure (Allakhverdiev *et al.* 2008, Lefsrud *et al.* 2008, Trojak and Skowron 2017, Jensen *et al.* 2018). If the light intensity is too high, this leads to elevated contents of ROS, which, in addition to their damaging effects, can perform signalling functions. In response to increased ROS formation, various low-molecular-mass antioxidants are synthesised and the activity of antioxidant enzymes is increased. Enzymatic antioxidants, carotenoids, phenolic compounds, including anthocyanin, play a significant role in ROS detoxification (Ouzounis *et al.* 2015, Mosadegh *et al.* 2018, Fan *et al.* 2019).

Carotenoids belong to the group of hydrophobic substances that are localized in chloroplasts as part of light-harvesting complexes (Rodriguez-Amaya 2019). Carotenoids, as accessory pigments, absorb light in the short-wavelength region of the spectrum, and then the absorbed energy is transferred to chlorophyll (Chl) (Frank and Cogdell 1996). Carotenoids are considered effective quenchers of singlet oxygen ($^1O_2$) and triplet Chls through a physical mechanism that involves the transfer of excitation energy leading to thermal deactivation. Additionally, leaf carotenoids can quench $^1O_2$ by a chemical mechanism involving their oxidation (Ramel *et al.* 2012). Carotenoids not only participate in the light reactions of photosynthesis and protect the PA from light excess but also support the structural stability of pigment-protein light-harvesting complexes (Yabuzaki 2017, Duarte *et al.* 2019). The constitutive carotenoid content in plants, as a majority

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Abbreviations: F_v/F_m – maximum quantum yield of PSII; HIL – high-intensity light; LED – light-emitting diode; PA – photosynthetic apparatus; q_L – fraction of PSII open centres; ROS – reactive oxygen species; TBARs – thiobarbituric acid reactive substances; WT – wild type; $Y_{(II)}$ – effective quantum yield of PSII; $Y_{(NO)}$ – quantum yield of nonregulated nonphotochemical energy dissipation in PSII; $Y_{(NPQ)}$ – quantum yield of regulated nonphotochemical energy dissipation in PSII.
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of plant metabolites, is determined by the genome, but their quality and quantity are affected by external factors, primarily the light intensity and spectral composition. It was shown that, in *Ginkgo biloba* and *Erigeron breviscapus* plants, the carotenoid content decreases with shading and rises with increasing light intensity (Frank and Cogdell 1996).

Anthocyanins are water-soluble pigments and the final products of the late stage of the flavonoid biosynthetic pathway (Zhang *et al.* 2018). They are located in plant cells mainly in peripheral tissues, such as the upper mesophyll, which is most susceptible to HIL (Trojak and Skowron 2017). In plants, anthocyanins, similar to all flavonoids, are among the protective compounds that function as an optical filter, which prevents increased ROS production in the photosynthetic electron transport chain (Mierziak *et al.* 2014, Khan *et al.* 2018, De Keyser *et al.* 2019). The anthocyanin content varies significantly in different plant species, and their protection depends on various factors (Shoji *et al.* 2010, Ali and Abbasi 2014, Rodriguez-Amaya 2019). Anthocyanins protect PA from photons in excess that could be absorbed by the pigment of the antenna complex, while red leaves absorb plenty light because of nonphotosynthetic pigment (*e.g.* anthocyanin), thus, their photosynthetic tissues can receive fewer quanta than green leaves (Gould *et al.* 2000, Manetas 2006). Intensive accumulation of anthocyanin [up to 2.2–2.5 mg g^{-1}(FM)] was detected by periodically irradiating red-leaved basil with light [120 μmol(photon) m^{-2} s^{-1}, 12 h each day] (Lobiuc *et al.* 2017). Additionally, the anthocyanin-rich *Arabidopsis thaliana* mutant *pap1-D* was less susceptible to PA damage than WT plants when exposed to HIL [800 μmol(photon) m^{-2} s^{-1}, 2 h] at 10°C (Gould *et al.* 2018). Anthocyanins are believed to be active in photoprotection when plants are subjected to prolonged exposure to the intense light of fluorescent lamps or natural sunlight at low temperatures when the capacities of other photoprotective mechanisms are exhausted (Berry and Björkman 1980, Gould *et al.* 2018, De Keyser *et al.* 2019). The anthocyanin content depends on the spectral characteristics of the light. A significant increase in the anthocyanin content was observed when growing *Guzmania* and *Pelargonium* under LEDs [40–100 μmol(photon) m^{-2} s^{-1}, 14 d] in various combinations of blue and red light compared with plants grown under fluorescent lamps (Owen and Lopez 2017, De Keyser *et al.* 2019).

An important role in plant photoprotection is played by numerous phenolic compounds that can inactivate free radicals and protect cells from ROS action (Carvalho *et al.* 2016, Kalisz *et al.* 2016, Masondo *et al.* 2019). When studying plants under UV radiation, shading, and water deficiency, an increase in the total phenolic content, including the total flavonoid content, inhibition of growth processes, and increased resistance to various stress factors (Javanmardi *et al.* 2003, Bidel *et al.* 2007, Cheynier *et al.* 2013, Mosadegh *et al.* 2018) were observed. The main sites of their biosynthesis are plastids, mitochondria, and the endoplasmic reticulum, and the localization sites are vacuoles, cell wall, nucleus, and chloroplasts (Cheynier *et al.* 2013).

In the work of Tattini *et al.* (2014), the effect of high-intensity sunlight in the range of 600–2,000 μmol(photon) m^{-2} s^{-1} on the green- and purple-leaved *O. basilicum* varieties was studied. These authors concluded that at high light the purple-leaved is more tolerant than green-leaved variety, as metabolic cost of photoprotective mechanisms is low and the increase in biomass production is high passing from shade to full sun, and also that epidermal coumaroyl anthocyanins have a tremendous impact on the response mechanisms to high light stress, but they are unusual in being effective in absorbing mostly UV-B than green-yellow solar wavelengths. On the other hand, sunlight is not optimal for the growth of most plants and contains a lot of infrared radiation, which causes heating and complicates studying the mechanism of adaptation to HIL separately from temperature. Hosseini *et al.* (2019) showed that in both purple and green basil varieties, combination of red and blue LEDs (70 red:30 blue) caused favorable growth, pigmentation, and Chl fluorescence parameters of basil plants [250 ± 10 μmol(photon) m^{-2} s^{-1}]. Based on this we tried to implement a one-way experiment model to determine how different varieties of basil adapt to the prolonged exposure to the most suitable light for them separately from the temperature influence. For this, we used narrow-band diodes without IR and UV wavelengths, along with a collecting lens and cooling phytotron boxes.

The existence of *O. basilicum* varieties with different accumulations of anthocyanin and carotenoids makes them a convenient model system to assess the ability of the PA to adapt to HIL, as well as to establish the role of anthocyanin and carotenoids in these processes. In this regard, studies of the species-specific mechanisms of plant adaptation using various strategies to avoid oxidative stress caused by HIL are of interest. Thus, we aimed to investigate the PA adaptation of purple- and green-leaved *O. basilicum* to long-term growth at HIL [300–1,350 μmol(photon) m^{-2} s^{-1}].

Materials and methods

Plant growth and experimental design: We investigated *Ocimum basilicum* L., an annual medicinal and spicy aromatic plant of the Lambaceae (Lamiaceae) family. Two varieties of basil, lemon basil (*O. basilicum* × *O. americanum)* and 'Rubin' basil (*O. basilicum* 'Rubin'), contrasting in leaf colour, were used in the study. Basil seeds were sown in perlite and, at the stage of the second pair of true leaves, the seedlings were transferred to a Hoagland media (Hoagland and Arnon 1950*)* and were grown in a climatic chamber in conditions of 12/12-h light/dark regime, 150 ± 10 μmol(photon) m^{-2} s^{-1} (*Philips L36W/765* fluorescent lamps, Poland), 22 ± 3°C, relative air humidity of 65 ± 5%. After four weeks, the control plants were fixed and primary analyses were performed. The remaining plants were placed in a phytotron chamber at 22 ± 3°C and a relative air humidity of 65 ± 5%, equipped with white LEDs (100 W, *EPISTAR*, Taiwan), emission spectra in two maxima [blue spectral regions (450 nm):red spectral regions (660 nm) = 1:3] [the spectral characteristics of the light sources were determined using the *USB2000*

spectrometer (*OceanOptics*, USA); Fig. 1S, *supplement*], a colour temperature of 3,300 K, and a collecting lens (d = 50 mm), providing light intensities of 300, 550, and 1,350 μmol(photon) m^{-2} s^{-1} (*Li-250A*, *Li-COR*, USA) and eliminating the heat influence. In experimental plants, every 7 d for three weeks, physiological, biochemical, and photosynthetic parameters were measured. Some plants were fixed in liquid nitrogen and stored at –70°C until other analyses were performed.

Determination of the photochemical activity: The fluorescent parameters were measured using an *IMAGING-PAM* fluorometer (*Walz*, Germany) on plants adapted to the dark (30 min). After a pulse of saturating light, the leaves were kept for 1 min in the dark, and then they were exposed to actinic light for 5 min, followed by saturating light pulses during which the parameters were measured. The areas of interest were selected on the leaf so that measurements from each pixel of this zone provided averaged values over the entire leaf surface. During one measurement, three leaves of the second tier were used. The leaves were placed in front of the camera at a distance of 7 cm, and then six measured zones were allocated on each leaf. The shape of the zones was circular (radius: 0.86 mm, area: 2.35 mm^2). The measuring light, actinic light, and saturating pulses were provided by blue LEDs (450 nm). The intensity of the actinic blue light was 220 μmol(photon) m^{-2} s^{-1}. The intensity of the measuring light was 0.5 μmol(photon) m^{-2} s^{-1}. The intensity and duration of the saturation pulse were 5,000 μmol(photon) m^{-2} s^{-1} and 800 ms, respectively. The primary data processing and parameter calculations were performed using *Imaging Win v. 2.41a* software (*Walz*, Germany) and the following formulas (Kramer *et al.* 2004, Klughammer and Schreiber 2008, Goltsev *et al.* 2016): (*1*) F_v/F_m, maximal PSII quantum yield. It is measured similarly to the $Y_{(II)}$ but only for dark-adapted leaves. $F_v/F_m = (F_m - F_0)/F_m$, where F_m is the maximal fluorescence yield of the dark-adapted sample when all the PSII centres are closed and F_0 is the minimal fluorescence yield of dark-adapted samples when all the PSII centres are open. (*2*) $Y_{(II)}$, effective PSII quantum yield. This parameter constitutes the fraction of energy that is photochemically converted in the PSII, $Y_{(II)} = (F_m' - F_s)/F_m'$, where F_m' is the maximal fluorescence yield of light-adapted samples and F_s is the relative steady-state fluorescence yield. (*3*) q_L, a parameter estimating the fraction of PSII open centres. It is based on a lake model for photosynthetic unity (Kramer *et al*. 2004); $q_L = [(F_m' - F_s)/(F_m' - F'_0)] \times (F_0'/F_s)$, where F_0' is minimal fluorescence yield of light-adapted samples. (*4*) NPQ, nonphotochemical fluorescence quenching, $NPQ = F_m/F_m' - 1$. (*5*) $Y_{(NO)}$, the quantum yield of nonregulated energy dissipation in PSII. The yield reflects the fraction of the energy that is passively dissipated in the form of heat and fluorescence, $Y_{(NO)} = 1/[NPQ + 1 + q_L \times (F_m/F_0 - 1)]$. (*6*) $Y_{(NPQ)}$, the quantum yield of regulated energy dissipation in PSII. The fraction of energy dissipated in the form of heat *via* the regulated photoprotective mechanism, $Y_{(NPQ)} = 1 - Y_{(II)} - Y_{(NO)}$.

Pigment and water content: The biomass of the above-ground part was determined using the gravimetric method to an accuracy of 0.01 mg (*OHAUS Scout STX*, USA). The samples were then dried for three days (60°C) to a constant mass and then weighed again.

The photosynthetic pigment (Chls *a* and *b*, Car) was determined using the Lichtenthaler (1987) method. The pigments were extracted with 80% acetone in 3–4 multiple repetitions until the samples were completely bleached at 4°C. The optical density of the extracts was determined at wavelengths of 470, 648, and 663 nm using a spectrophotometer (*Genesys 10 UV*, *Thermo Fisher Scientific*, USA).

Anthocyanin and total free phenol content: The anthocyanin content was determined using the Giusti and Wrolstad (2001) method. Cyanidin solutions (*Gee Lawson Chemicals*, UK) were used to make the calibration curve 1.25–20.0 μg ml^{-1}. The plant material was suspended in acid/alcohol mixture (97 ml of 96% ethanol + 3 ml of concentrated HCl). The content of anthocyanin was determined at 550 nm (*Genesys 10 UV*, *Thermo Fisher Scientific*, USA).

The total phenol compounds from plant material were extracted with 96% ethanol. Content was determined using the Folin-Ciocalteu reagent according to the Singleton *et al*. (1999) method at 725 nm (*Genesys 10 UV*, *Thermo Fisher Scientific*, USA). Gallic acid (*Sigma*, Germany) was used as a standard solution for calibration at a concentration of 50–450 μg ml^{-1}.

H_2O_2 content: The content of hydrogen peroxide was determined spectrophotometrically using the FOX method (ferrous oxidation-xylenol orange method) (Bellincampi *et al.* 2000), which is based on the oxidation of Fe^{2+} by hydrogen peroxide to Fe^{3+} (catalysed by sorbitol) with the formation of coloured compounds. To this end, 70–80 mg of plant tissue was extracted with chilled acetone and centrifuged for 10 min at 12,000 × *g*. An equal volume of FOX reagent (*Gee Lawson Chemicals*, UK) was added to the obtained extract and kept for 45 min at room temperature. A mixture of FOX reagent and acetone at a ratio of 1:1 was used as a control. The optical density was measured at a wavelength of 560 nm using a spectrophotometer (*Genesys 10 UV*, *Thermo Fisher Scientific*, USA).

TBARs content: TBARs were determined using the Heath and Packer (1968) method, based on the formation of a coloured complex of TBARs with thiobarbituric acid (*Sigma*, Germany) through heating. The concentration of TBARs was determined spectrophotometrically by measuring the optical density on a *Genesys 10 UV* spectrophotometer (*Thermo Fisher Scientific*, USA) at 532 and 600 nm wavelengths. As a TBARs standard 1,1,3,3-tetramethoxypropane (*Sigma*, Germany) in deionized water was used.

Statistical analysis: At least three independent biological experiments were carried out. The analysis of each point of each variant included six plants. Three leaves of the second and third tiers of plants formed in the new lighting conditions were used in the analyses. Data were represented

as mean ± SD. One-way analysis of variance (*ANOVA*) followed by *Duncan*'s method were performed using *SigmaPlot 12.3* (*Systat Software Inc*., USA). Different letters were used to represent significance at $p<0.05$.

Results

Biomass and water content: The aerial mass of both basil varieties increased with increasing light intensity (Table 1, Fig. 1). However, under all studied light conditions, the biomass accumulation was higher in green-leaved than that in purple-leaved plants. At 21 d, the mass of the aboveground part of green-leaved plants grown at a light intensity of 1,350 μmol(photon) m^{-2} s^{-1} increased 2.6 times, and the mass of purple-leaved plants increased, on average, 1.5 times compared with plants that were grown at 300 μmol(photon) m^{-2} s^{-1} (Table 1). A greater increase in green-leaved plants was also observed at other higher light intensities. The water content of the leaves of both basil varieties mostly decreased with increasing light intensity (Table 1).

Importantly, green-leaved plants were less susceptible to leaf dehydration than purple-leaved plants. At 21 d of growth at 1,350 μmol(photon) m^{-2} s^{-1}, the water content decreased by an average of 4% in the green-leaved plants and by an average of 22% in purple-leaved plants compared with the initial-point control values (Table 1).

Photochemical activity

Maximum quantum yield of PSII: The F_v/F_m in the green-leaved plants throughout the experiment remained high, within 0.77–0.80, and only decreased to 0.70 at 21 d at 1,350 μmol(photon) m^{-2} s^{-1} (Fig. 2). In purple-leaved plants, the basilic F_v/F_m values at 7 d at 550 and 1,350 μmol(photon) m^{-2} s^{-1} were slightly lower than that at 150 μmol(photon) m^{-2} s^{-1} (approximately 0.74), however, at 14 and 21 d at 550 μmol(photon) m^{-2} s^{-1}, the F_v/F_m was close to the initial values (Fig. 2*A*).

Effective quantum yield of PSII: In green-leaved plants, at all the used light intensities, the $Y_{(II)}$ fluctuated within 0.5 ± 0.05, indicating the high efficiency of the PA. At 550 and 1,350 μmol(photon) m^{-2} s^{-1}, the plants quickly adapted to light conditions, maintaining a sufficiently high effective quantum yield. In purple-leaved plants, at 7 d, all the variants showed the same $Y_{(II)}$ levels as the control, however, under HIL, the $Y_{(II)}$ significantly increased. The purple-leaved plants grown at 550 μmol(photon) m^{-2} s^{-1} for 14 and 21 d were the most effective. The $Y_{(II)}$ value was lower than that of the control at 21 d under 1,350 μmol(photon) m^{-2} s^{-1} (Fig. 2*B*). The distribution of the $Y_{(II)}$ value over the leaf in plants grown at two light intensities [550 and 1,350 μmol(photon) m^{-2} s^{-1}] is shown in Fig. 3. In all cases, a high and uniform distribution of $Y_{(II)}$ over the leaf was observed, excluding purple-leaved plants grown at 1,350 μmol(photon) m^{-2} s^{-1}. Along with areas where the PA works efficiently (Fig. 3, in green), several areas had a low effective quantum yield (in red). A comparison of q_L revealed the same patterns: a high q_L value in green-leaved plants and a very low q_L value in purple-leaved plants under 1,350 μmol(photon) m^{-2} s^{-1} but an intermediate q_L value at 550 μmol(photon) m^{-2} s^{-1} in purple-leaved plants. At the same time, a pronounced uneven distribution of this value over the leaves was found (Fig. 3). This parameter is an indicator of a Q_A redox state (Kramer *et al.* 2004). Hence, the redox state of Q_A has heterogeneous distribution within the leaf.

Nonphotochemical chlorophyll fluorescence quenching: The NPQ in green-leaved plants was low (0.2–0.3) and on 21th day it decreased from 0.3 at 550 μmol(photon) m^{-2} s^{-1} to 0.2 at 1,350 μmol(photon) m^{-2} s^{-1}, whereas in purple-leaved plants it increased from 0.2 at 550 μmol(photon) m^{-2} s^{-1} and 0.6 at 1,350 μmol(photon) m^{-2} s^{-1}.

The $Y_{(NPQ)}$ level in green-leaved plants increased in the variant grown at 550 μmol(photon) m^{-2} s^{-1} at 14 and 21 d compared with that at 7 d (Fig. 2*C*). However, in the variant grown at 1,350 μmol(photon) m^{-2} s^{-1}, $Y_{(NPQ)}$ was at the control level. The green-leaved $Y_{(NO)}$ value increased at 550 and 1,350 μmol(photon) m^{-2} s^{-1} except for that of the variant grown at 550 μmol(photon) m^{-2} s^{-1} at 21 d (Fig. 2*D*). In purple-leaved plants, the $Y_{(NPQ)}$ value in the control was higher than that in green-leaved plants. On the 14th day of the experiment, this parameter decreased both at 550 and 1,350 μmol(photon) m^{-2} s^{-1} with a subsequent increase. The highest $Y_{(NPQ)}$ values for purple-leaved plants were observed at 1,350 μmol(photon) m^{-2} s^{-1} on day 21 (Fig. 2*C*). The $Y_{(NO)}$ value at 7 d under 550 and 1,350 μmol(photon) m^{-2} s^{-1} was at the control level. A gradual decrease in $Y_{(NO)}$ was then observed, and, by the end of the experiment, in the 1,350 μmol(photon) m^{-2} s^{-1} variant reached the lowest values (Fig. 2*D*). Comparison of the $Y_{(NO)}$ values in the two types of plants on the 21st day of cultivation at two light intensities revealed a uniform distribution of this parameter over the leaf surface, but $Y_{(NO)}$ was higher in a dose-dependent manner (Fig. 3). Conversely, in purple-leaved plants, $Y_{(NO)}$ decreased and $Y_{(NPQ)}$ increased at higher light intensities. Heterogeneous distribution of $Y_{(NPQ)}$ over the leaf surface was observed (Fig. 3).

Pigment content: Comparison of the Chl *a* and *b* accumulation dynamics under prolonged intense light revealed varietal differences (Table 1). In green-leaved basil plants, the content of Chl *a* and *b* in all experimental variants did not decrease below the initial level and the highest values of the Chl content were observed at light intensities of 300 and 550 μmol(photon) m^{-2} s^{-1} (Table 1). In the purple-leaved basil plants, the Chl *a* and *b* content at 300 and 550 μmol(photon) m^{-2} s^{-1} increased for 14 d, however, at 21 d at 550 μmol(photon) m^{-2} s^{-1}, a decrease in the Chl contents was observed compared with that of the initial-point control. A significant decrease in the contents of Chl *a* and *b* were observed under 1,350 μmol(photon) m^{-2} s^{-1} throughout the entire experimental period, likely indicating the presence of a photoinhibition process in plants (Table 1).

The carotenoid content in the leaves of the control plants of both basil varieties did not significantly differ

Table 1. Light intensity influence on the plant growth process: aboveground fresh mass, water content, chlorophyll (*a*+*b*) and total free phenolic content in the leaves of green-leaved and purple-leaved basil over a growing period of 7–21 d. The plants were grown at a light intensity of 150 µmol(photon) m^{-2} s^{-1} (fluorescent lamps: initial-point control), 300, 550, and 1,350 µmol(photon) m^{-2} s^{-1} (LEDs). Data were represented as mean ± SD, $n = 6$. One-way analysis of variance (*ANOVA*) followed by *Duncan*'s method were performed. *Different letters* were used to represent significance at $p<0.05$.

	Light intensity [µmol(photon) $m^{-2}s^{-1}$]	Aboveground fresh mass [g]		H_2O content [%]		Chl (*a*+*b*) [mg g^{-1}(DM)]		Total phenolic compounds [µmol g^{-1}(FM)]	
		green-leaved	purple-leaved	green-leaved	purple-leaved	green-leaved	purple-leaved	green-leaved	purple-leaved
Initial point	150	1.42 ± 0.13	0.88 ± 0.05	91.74 ± 4.32	93.44 ± 4.32	10.47 ± 0.42	12.69 ± 0.54	6.40 ± 0.32	6.12 ± 0.33
7 d	150	1.53 ± 0.14^{d}	1.23 ± 0.06^{b}	91.83 ± 4.33^{a}	93.35 ± 4.29^{a}	11.67 ± 0.47^{c}	13.11 ± 0.47^{b}	6.58 ± 0.35^{b}	6.82 ± 0.34^{d}
	300	2.14 ± 0.15^{c}	1.62 ± 0.08^{a}	92.03 ± 3.07^{a}	93.02 ± 3.12^{a}	12.12 ± 0.57^{b}	13.52 ± 0.66^{a}	6.43 ± 0.34^{b}	7.41 ± 0.35^{c}
	550	3.42 ± 0.18^{b}	1.84 ± 0.09^{a}	92.13 ± 4.37^{a}	92.87 ± 4.53^{a}	15.21 ± 0.72^{a}	13.92 ± 0.72^{a}	7.42 ± 0.37^{a}	8.52 ± 0.44^{b}
	1,350	4.51 ± 0.24^{a}	2.06 ± 0.12^{a}	91.34 ± 4.56^{a}	91.43 ± 4.52^{b}	15.34 ± 0.76^{a}	5.77 ± 0.28^{c}	9.73 ± 0.48^{a}	10.37 ± 0.56^{a}
14 d	150	2.45 ± 0.17^{d}	1.48 ± 0.03	92.02 ± 3.32^{a}	93.14 ± 2.32^{a}	12.45 ± 0.43^{b}	13.69 ± 0.54^{b}	6.90 ± 0.32^{c}	7.61 ± 0.33^{d}
	300	3.61 ± 0.21^{c}	2.80 ± 0.15^{b}	93.26 ± 4.21^{a}	92.98 ± 3.21^{a}	14.95 ± 0.61^{a}	14.80 ± 0.72^{a}	7.47 ± 0.37^{c}	10.87 ± 0.56^{c}
	550	5.78 ± 0.38^{b}	2.82 ± 0.16^{b}	92.44 ± 5.18^{a}	92.51 ± 4.61^{a}	15.72 ± 0.62^{a}	13.77 ± 0.67^{b}	8.17 ± 0.43^{b}	13.05 ± 0.82^{b}
	1,350	9.35 ± 0.46^{a}	3.10 ± 0.17^{b}	90.35 ± 4.27^{b}	89.79 ± 4.51^{b}	14.82 ± 0.73^{a}	5.20 ± 0.23^{c}	12.47 ± 0.63^{a}	15.03 ± 0.76^{a}
21 d	150	3.42 ± 0.33^{d}	2.88 ± 0.26^{c}	92.74 ± 4.12^{a}	92.44 ± 3.32^{a}	14.57 ± 0.42^{b}	14.69 ± 0.54^{b}	7.40 ± 0.32^{d}	10.71 ± 0.33^{d}
	300	5.57 ± 0.36^{c}	4.34 ± 0.22^{b}	93.24 ± 4.24^{a}	82.45 ± 4.12^{b}	17.64 ± 0.84^{a}	15.18 ± 0.74^{a}	9.73 ± 0.53^{c}	11.63 ± 0.54^{c}
	550	10.35 ± 0.52^{b}	6.10 ± 0.32^{a}	91.21 ± 4.53^{b}	78.39 ± 4.11^{c}	16.93 ± 0.78^{a}	10.73 ± 0.52^{c}	11.33 ± 0.56^{b}	13.03 ± 0.83^{b}
	1,350	14.26 ± 0.73^{a}	6.44 ± 0.35^{a}	87.84 ± 4.41^{c}	71.08 ± 3.62^{d}	13.08 ± 0.64^{c}	3.77 ± 0.17^{d}	14.73 ± 0.76^{a}	14.20 ± 0.69^{a}

and averaged 0.73 mg g^{-1}(DM) (Fig. 4*A*,*B*). Exposure of the plants at varying light intensities revealed varietal differences in the dynamics of changes in the carotenoid content. In green-leaved plants, the carotenoid content was enhanced with increasing time exposure, at 21 d, at 300, 550, and 1,350 µmol(photon) m^{-2} s^{-1}, the carotenoid content exceeded that of the initial control values by an average of 1.8, 1.6, and 1.4 times, respectively (Fig. 4*A*) The dynamics of carotenoid accumulation in green-leaved basil at light intensities of 300 and 550 µmol(photon) m^{-2} s^{-1} increased, a slight decrease was observed only at 1,350 µmol(photon) m^{-2} s^{-1} (Fig. 4*A*). In purple-leaved basil plants, with an increase in the light intensity and duration of light exposure, inhibition of carotenoid synthesis occurred compared with the initial-point control value (Fig. 4*B*). The decrease in the carotenoid content in purple-leaved basil on the 21st day of the experiment at light intensities of 300, 550, and 1,350 µmol(photon) m^{-2} s^{-1} averaged 7, 17, and 66%, respectively, compared with that of the initial-point control (Fig. 4*B*).

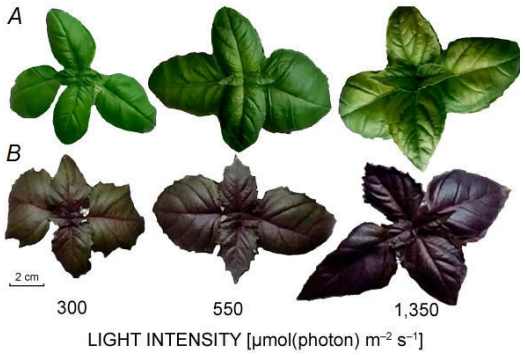

Fig. 1. Leaf images of green-leaved (*A*) and purple-leaved basil (*B*) plants grown on hydroponics for 14 d at different light intensities: 300, 550 and 1,350 µmol(photon) m^{-2} s^{-1}.

Anthocyanin content: The constitutive anthocyanin content in green-leaved basil leaves averaged 0.7 µg g^{-1}(FM) (Fig. 4*C*,*D*). Because of green-leaved basil exposure to 300 and 1,350 µmol(photon) m^{-2} s^{-1} for 21 d, the anthocyanin contents in the leaves increased by an average of 20 and 35%, respectively (Fig. 4*C*). The leaves of purple basil

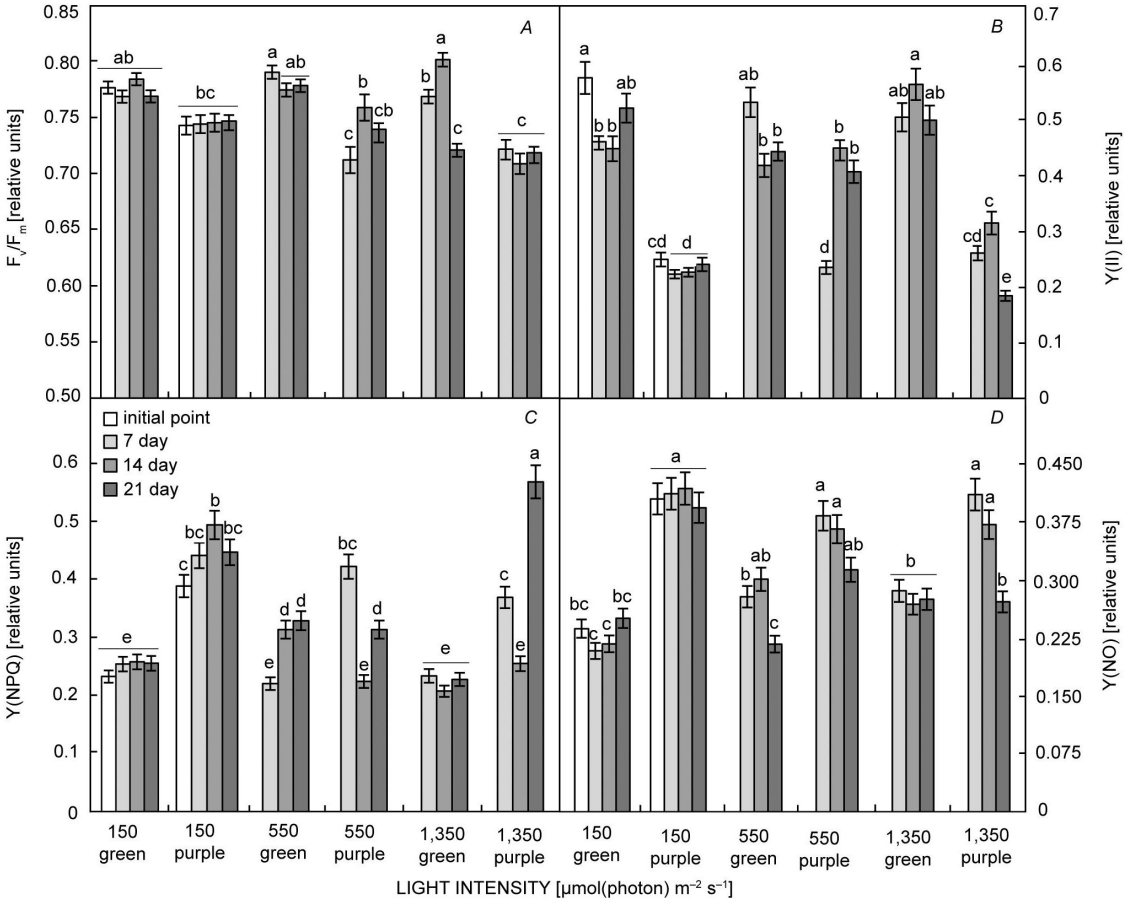

Fig. 2. Effect of light intensity and exposure time (7, 14, 21 d) on the PSII maximum quantum yield (F_v/F_m) (*A*), effective quantum yield ($Y_{(II)}$) (*B*), quantum yields of regulated ($Y_{(NPQ)}$) (*C*), and nonregulated ($Y_{(NO)}$) (*D*) energy dissipation in green-leaved and purple-leaved basilic leaves. The plants were grown for 21 d at different light intensities: 150 µmol(photon) m^{-2} s^{-1} (initial point), 550, and 1,350 µmol(photon) m^{-2} s^{-1}. Data were represented as mean ± SD, $n = 6$. One-way analysis of variance (*ANOVA*) followed by *Duncan*'s method were performed. *Different letters* were used to represent significance at $p<0.05$.

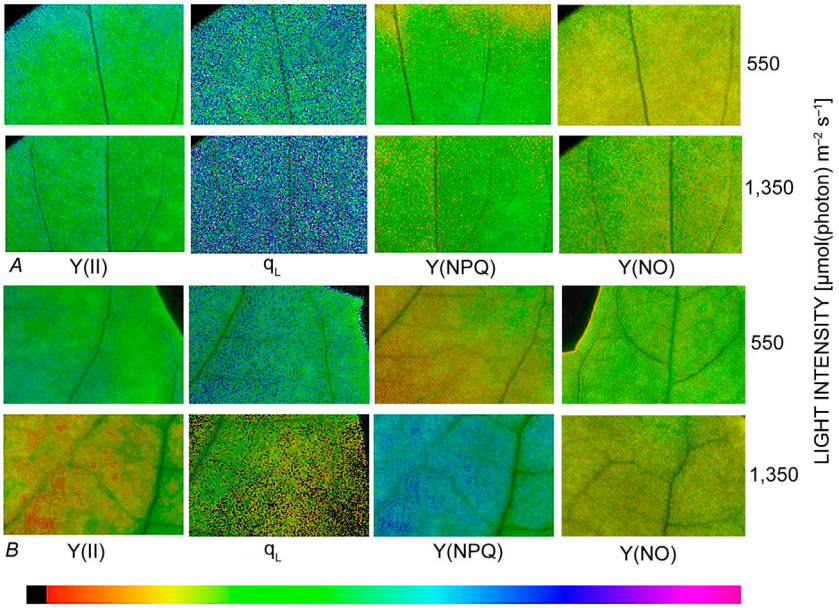

Fig. 3. Typical fluorescence images of the effective quantum yield of PSII ($Y_{(II)}$), fraction of open reaction centres of the PSII (q_L), quantum yields of regulated ($Y_{(NPQ)}$), and nonregulated ($Y_{(NO)}$) nonphotochemical quenching in leaves green-leaved (*A*) and purple-leaved (*B*) basil after exposure of plants for 21 d at two light intensities: 550 and 1,350 μmol(photon) m^{-2} s^{-1}. All images were normalised to the provided false colour panel. The display of pixel values is based on a scale of false colours, ranging from black (0–0.04) to red, yellow, green, blue, and violet (ends with 1).

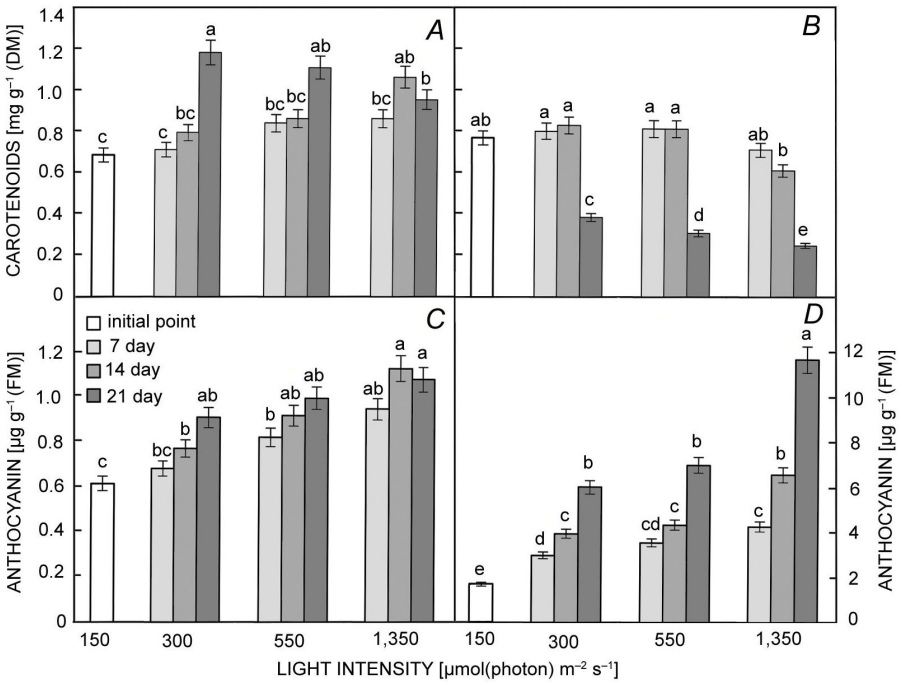

Fig. 4. Effect of light of different intensities and exposure time (7, 14, and 21 d) on the content of carotenoids (*A*,*B*) and anthocyanin (*C*,*D*) in the leaves of green-leaved (*A*,*C*) and purple-leaved basil (*C*,*D*) plants grown at various light intensities: 150 (initial point), 300, 550, and 1,350 μmol(photon) m^{-2} s^{-1}. Data were represented as mean ± SD, $n = 6$. One-way analysis of variance (*ANOVA*) followed by *Duncan*'s method were performed. *Different letters* were used to represent significance at $p<0.05$.

with an intense violet colour were characterized by a high content of anthocyanin that averaged 1.8 μg g^{-1}(FM) in the control plants (Fig. 4*D*). The increase in light intensity during the cultivation of purple-leaved basil experimental plants was accompanied by a significant increase in the anthocyanin content in all variants compared with that of green-leaved basil (Fig. 4*D*). At a light intensity of 1,350 μmol(photon) m^{-2} s^{-1}, the anthocyanin content in purple-leaved basil leaves at 14 and 21 d increased 3.5 and 6 times, respectively, compared with that of the initial-point control (Fig. 4*D*).

Total free phenolic content: Regarding the total free phenol content, the green-leaved and purple-leaved basil at the beginning of the experiment did not differ significantly and the free phenolic content was in the range of 5–7 μmol g^{-1}(FM) (Table 1). HIL irradiation caused an increase in the free phenol content in the leaves. At 14 d of exposure at 1,350 μmol(photon) m^{-2} s^{-1}, the phenolic content increased on average two times in green-leaved basil and 2.5 times in purple-leaved basil compared with the initial-point control values [150 μmol(photon) m^{-2} s^{-1}] (Table 1). At 21 d, a further increase in the total free phenol

content was observed in the leaves of green basil grown at 300, 550, and 1,350 µmol(photon) m^{-2} s^{-1} compared with the initial-point control by averages of 1.5, 2.8, and 3.3 times, respectively (Table 1). An increase in exposure time from 14 to 21 d did not significantly affect the content of the phenolic compounds in purple-leaved basil leaves.

TBARs content: The TBARs content in the leaves of both varieties increased most significantly during the first week of exposure and was dose-dependent (Fig. 5*A*,*B*). At a light intensity of 1,350 µmol(photon) m^{-2} s^{-1} in the green-leaved basil, the TBARs content increased on average 2 times over 7 d (Fig. 5*A*), and an average of 3 times in the purple-leaved basil leaves, compared with control plants (Fig. 5*B*). On the 21st day of the experiment, in both varieties, at light intensities of 300, 550, and 1,350 µmol(photon) m^{-2} s^{-1}, the TBARs content decreased relative to the 7th day of exposure (Fig. 5*A*,*B*), possibly indicating the adaptation of plants to the lighting regime. At 21 d, the TBARs content decreased in both varieties compared with that on the 7th d of exposure (Fig. 5*A*,*B*).

H_2O_2 content: In the leaves of the control plants of both varieties, the H_2O_2 content was similar and averaged 0.5–0.8 µmol g^{-1}(FM) (Fig. 5*C*,*D*). With increasing light intensity and exposure duration, enhancement in the H_2O_2 content of both varieties was observed. However, the varieties significantly differed from each other in the dynamics of hydrogen peroxide content. The content of H_2O_2 in the leaves of green-leaved basil increased four times at 21 d of exposure at a light intensity of 1,350 µmol(photon) m^{-2} s^{-1}, and that in the purple-leaved basil increased on average three times, compared with that of the initial-point control (Fig. 5*C*,*D*).

Discussion

First, we investigated the relationship between the changes in the photosynthetic parameters during long-term HIL cultivation and changes in the carotenoid (Fig. 4*A*,*B*) and anthocyanin (Fig. 4*C*,*D*) content. We must understand how these pigments can support growth and PA activity under photoinhibition. Carotenoids and anthocyanin, which are found in different cell compartments, can perform common photoprotective functions, however, their mechanisms of action differ (Yin *et al.* 2015, Zhang *et al.* 2018, Kumar and Kim 2019).

Regarding the carotenoid content, the control plants, green-leaved basil and purple-leaved basil did not differ significantly (Fig. 4*A*,*B*). An increase in the carotenoid content in response to the HIL action is an important adaptive mechanism present in plants and algae (Vo *et al.* 2017, Wurtzel 2019). However, such a relationship is not always unambiguous. Thus, in the leaves of green-leaved basil, the carotenoid content increased markedly as the plants were exposed to light (Fig. 4*A*). By contrast, under the same conditions in purple-leaved basil, the carotenoid content decreased (Fig. 4*B*). In green-leaved basil plants, carotenoids appear to be an effective protective mechanism for PA protection, however, in purple-leaved basil plants,

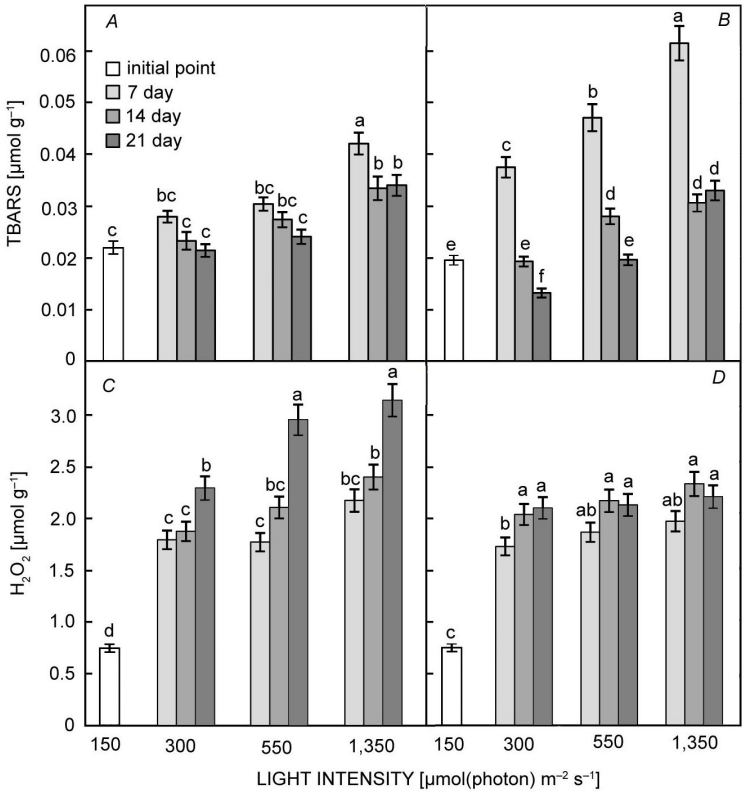

Fig. 5. Effect of different light intensities on the content of thiobarbituric acid reactive substances (TBARs) (*A*,*B*) and H_2O_2 (*C*,*D*) in the leaves of green-leaved basil (*A*,*C*) and purple-leaved basil (*B*,*D*) plants grown under different exposure durations: 7, 14, and 21 d. Light intensities: 150 (initial point), 300, 550, and 1,350 µmol(photon) m^{-2} s^{-1}. Data were represented as mean ± SD, n = 6. One-way analysis of variance (*ANOVA*) followed by *Duncan*'s method were performed. *Different letters* were used to represent significance at $p<0.05$.

this mechanism is less effective (Fig. 4*A*,*B*).

In sunlight, purple-leaved basil plants are more successfully adapted to brief and intense light of 2,000 μmol(photon) m^{-2} s^{-1} due to the presence of UV in sunlight spectrum (Tattini *et al*. 2014). In the purple plant, epidermal coumaroyl anthocyanins play the role of an optical filter that prevents the penetration of UV into the mesophyll (Tattini *et al.* 2014). In purple-leaved basil plants, we observed a decrease in the pigments content and in the $Y_{(II)}$ together with the increase in anthocyanin and TBARs (Figs. 2–4, Table 1). Landi *et al* (2014) showed that the isolated protoplasts of purple-leaved basil were more adapted to intense sun light, more likely due to the high content of ascorbate and glutathione than anthocyanin. We did not determine the content of these antioxidants, however, with respect to other studied parameters in purple-leaved basil at HIL, its protective resources were exhausted earlier and lost water in the end of the experiment was observed (Table 1).

With an increase in light intensity, green-leaved basil plants showed extended internodes, while purple-leaved, on the contrary, showed a decrease in the length of internodes and biomass (data not shown). The green-leaved PA is likely more plastic and, despite the increase in H_2O_2 content, continues grow successfully, maintaining a high content of pigments and an effective quantum yield (Fig. 2, Table 1).

It has been shown earlier that intense sunlight caused a stress phenotype in green basil (Tattini *et al*. 2014). These authors suggested that the absence of photomorphogenetic responses in purple-leaved plants growing under high UV irradiance was due to coumaroyl derivatives of cyanidin 3-O-glycosides distributed in adaxial and abaxial epidermis. The concentration of coumaroyl anthocyanin in purple-leaved basil is enhanced in sun leaves, thus constituting an effective shield (Edwards *et al.* 2008) against the penetration of UV wavelengths in the shade, not only in full sun (Tattini *et al.* 2014). It has been suggested that anthocyanin protect plants from UV more than from light of other wavelengths (Terashima *et al.* 2005).

It is known that an increase in the anthocyanin content is a widespread mechanism of plant adaptation to HIL (Shoji *et al.* 2010, Yin *et al.* 2015, Owen and Lopez 2017, Gould *et al.* 2018, Liu *et al.* 2018). In our experiments, the constitutive content of anthocyanin in purple basilic leaves was two times higher than that in green-leaved basil (Fig. 4*C*,*D*). Growth under intense light for 21 d led to slight enhancement in the anthocyanin content in green-leaved plants (Fig. 4*C*) and the almost six-fold increase in the anthocyanin content in purple-leaved basil compared with the initial-point control (Fig. 4*D*). In leaves concomitantly exposed to high temperature (> 33°C) and high sunlight irradiance [> 1,700 μmol(photon) m^{-2} s^{-1}] anthocyanin preserved photosynthesis in excess light (Tattini *et al.* 2014). In contrast to this, our experiments without high temperature effect showed that prolonged exposure to LEDs HIL reduces both the protective properties of anthocyanin and probably antioxidant abilities of purple-leaved basil mesophyll cells (Table 1). The effective PSII quantum yield $Y_{(II)}$ in purple-leaved basil increased only at a moderate light intensity of 550 μmol(photon) m^{-2} s^{-1}, however, at a higher intensity, $Y_{(II)}$ was noticeably lower than in green-leaved basil (Fig. 2).

In green- and purple-leaved basils, biomass was significantly different depending on light intensity (Table 1). It is known that flavonoid-rich ecotypes display reduced biomass production with respect to their flavonoid-poor relatives (Hofmann and Jahufer 2011). Anthocyanin-rich mutants of *A. thaliana* grow worse compared to a wild type (Misyura *et al.* 2013). The biosynthesis of phenylpropanoids is energetically expensive in purple-leaved plants, but the accumulation of these metabolites may be of benefit under a wide range of stress (Hatier *et al.* 2013, Tattini *et al.* 2014). The authors suggested that purple-leaved plants will likely perform poorly in environments at limited sunlight availability, as the suite of morphoanatomical and biochemical traits leads to low ability of light interception and transmission in the leaf (Tattini *et al.* 2014). However, our data indicate that purple-leaved basil in low light conditions [150 μmol(photon) m^{-2} s^{-1}] only slightly exceeds growth parameters of green-leaved basil (Table 1). It is likely that the level of anthocyanin in low light is reduced, which allows increasing the penetration of light to the mesophyll (Fig. 4*C*,*D*).

The potential efficiency of PSII functioning is characterized by the maximum quantum yield (F_v/F_m), however, under light conditions, this activity is characterized by the effective quantum photochemical yield of $Y_{(II)}$ (Goltsev *et al.* 2016). This activity is also connected to the redox state of Q_A, which, assuming a lake model for PSII, is linearly related to the fluorescence parameter q_L (Kramer *et al.* 2004). Even under HIL, green-leaved basil can maintain a high effective quantum photochemical yield of PSII for three weeks, although F_v/F_m decreased slightly. Note, that the xanthophyll cycle is the main photoprotective mechanism functioning in photosynthetic organisms, which is associated with enzymatic reactions of epoxidation and deepoxidation of xanthophylls (Latowski *et al.* 2004). There is a close relationship between xanthophyll cycle and redox state of Q_A. So, investigations of *Chlorella vulgaris* culture demonstrated a correlation between increase in xanthophylls pool size and epoxidation of the pool pigments and redox state of Q_A, which was evaluated as $1 - q_P$ (Wilson and Huner 2000). However, Kramer (2004) using the lake model, suggested to use new parameter q_L (more suitable for terrestrial plants) instead of q_P and submitted new evaluation of redox state of Q_A as $1 - q_L$.

In purple-leaved plants, we indicated a decrease in q_L and hence increase in $1 - q_L$ that in our opinion corresponds to more reduced Q_A acceptor and lowered $Y_{(II)}$. Therefore, in purple plants, we can suggest an increase in xanthophylls pool size as a protective mechanism against high irradiance. Decline in value of $Y_{(II)}$ in purple-leaved plants and high level of this yield in green-leaved plants are also in accordance with the data on changes in NPQ, which was maximal in purple-leaved plants on the 21th day at light intensity of 1,350 μmol(photon) m^{-2} s^{-1} but

minimal in green-leaved plants.

Notably, no distinct correlation was found among the F_v/F_m, q_L, and $Y_{(II)}$ values likely because of the increased content of pigment in the leaves. The purple-leaved basil, despite the loss of water and a decrease in growth parameters in 21 d, retained the effective PSII quantum yield values close to that of the initial-point control [150 μmol(photon) m^{-2} s^{-1}]. However, $Y_{(II)}$ was noticeably lower than that of green-leaved basil, consistent with several areas demonstrating low PSII photochemical activity in purple-leaved plants (Fig. 3*B*). Moreover, the anthocyanin content and amount of free phenolic compounds were increased against the background of a decrease in H_2O_2 and TBARs (Fig. 5), possibly indicating a high adaptive potential of the plants and the success of the strategy of the accumulation of anthocyanin under HIL conditions during long-term cultivation.

It was hypothesized that an inherently low ability to activate xanthophyll cycle pigments may be a feature of purple-leaved plants (Nikiforou and Manetas 2010, Tattini *et al.* 2014). In contrast to the green-leaved plants, we showed that the HIL significantly reduced the amount of carotenoids in the purple-leaved basil on the 21st day of experiment even at 300 μmol(photon) m^{-2} s^{-1} (Fig. 4*A*,*B*). The reduced pool of xanthophyll cycle pigments is related to photoinhibitory damage in basil, as also reported previously in a range of species (Hughes *et al.* 2012, Tattini *et al.* 2014). The authors hypothesized, that NPQ operated less in purple-leaved than in green-leaved plants, to dissipate excess photons reaching the photosynthetic apparatus, because fewer photons reached the chloroplast (Hughes *et al.* 2012, Tattini *et al.* 2014). However, when the light intensity was extremely high and influenced for a longer time, the NPQ of purple-leaved basil significantly increased, and the effective quantum yield of PSII was reduced (Fig. 2), in contrast to the short-term action of intense sunlight. The observed enhanced energy dissipation $Y_{(NPQ)}$ in purple-leaved basil at HIL (compared with a control [150 μmol(photon) m^{-2} s^{-1}] is a possible protection mechanism during long-time growing to reduce PA damage . It is possible that purple-leaved basil is able to cope with short, but intense light, which includes UV, due to NPQ, while in the long time HIL treatment NPQ does not allow to transform all excess energy into heat, which leads to photodamage and water loss (Fig. 2, Table 1).

In addition to anthocyanin, other phenolic compounds are present in plant cells that are also sensitive to light conditions and are involved in photoprotection and photorepair (Csepregi and Hideg 2018, Nadeem *et al.* 2019). There is evidence that phenylpropanoids can play an even more important role than anthocyanin and carotenoids in basil plants under HIL exposure. Thus, carbon investment to phenylpropanoid biosynthesis was much greater in purple- than that in green-leaved basil in the shade, and only partially due to anthocyanin biosynthesis (Tattini *et al.* 2014). Light-induced accumulation of phenylpropanoids was much greater in green-leaved than in purple-leaved plants in response to high sunlight (Tattini *et al.* 2014). However, we did not observe such carotenoid enhancement in green-leaved basil as anthocyanin in purple-leaved plants (Fig. 4). The contents of carotenoids were close to each other at 550 and 1,350 μmol(photon) m^{-2} s^{-1} regardless of growing time.

In green basil, it is highly likely that PA is protected by other antioxidants. Nevertheless, the content of the total phenolic compounds was higher in purple basil, and only on 21 d both plants become equal in this parameter. Nazir *et al.* (2020) examined the antioxidant capacity of basil cell cultures under conditions of a different light spectrum. It turned out that the highest 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) activity of cell extracts was observed in the basilica cells exposed under the blue and white light. The free total phenolic and flavonoid contents were found to be positively correlated with antioxidant activities in cells (Ali and Abbasi 2014) and in whole plants of *O. basilicum* (Javanmardi *et al.* 2003, Carvalho *et al.* 2016, Nadeem *et al.* 2019). A similar correlation in our experiments was observed. HIL increased the total phenolic content in the leaves of both types of basil (Table 1), accompanied by a TBARs decrease at 14 and 21 d in both basil cultivars (Fig. 5*A*,*B*). The content of TBARs increased in both varieties in the first 7 d after the plants were kept at all light intensities which also were correlated with lowered content of phenolic compounds. This result suggests that both varieties adapt to HIL, however, the adaptation strategy of green basil is more successful, because it allows to maintain growth and $Y_{(II)}$ for a long time at high irradiance. In this case, the purple-leaved basil is likely to adapt *via* the accumulation of phenolic compounds and anthocyanin, while the green-leaved basil is adapted *via* the accumulation of carotenoids, phenolic compounds, and anthocyanin (partially). However, significant accumulation of anthocyanin in purple leaves on 21 d leads to decrease in photosynthetic activity likely due to essentially lowered light intensity reaching light-harvesting complexes in chloroplasts as well as reduced contents of photosynthetic pigments.

Conclusion: Our results are consistent with the concept of the important role of both anthocyanin and carotenoids in plant adaptation to HIL. We suggest that HIL of 1,350 μmol(photon) m^{-2} s^{-1} can be used for several weeks to enhance the nutritional and/or medicinal properties of basil. The optimal light intensity for growth is likely in the range of 550 μmol(photon) m^{-2} s^{-1} because most of the studied parameters of the two plant species converged at this light intensity. A high carotenoid content serves as a more effective photoprotective mechanism for the long-term cultivation of the photophilous basil plants on HIL using white LEDs. Carotenoids are also more effective for the biomass accumulation of the aerial part of basil in a wider range of light intensities. By contrast, anthocyanin provides a high level of $Y_{(II)}$ comparable to that of green-leaved basil at a moderate light intensity of 550 μmol(photon) m^{-2} s^{-1} compared with that of green-leaved basil at the same light intensity. Our results with LED illumination, in aspects, contradict those obtained in sunlight. Probably, the adaptive potential of the purple basil is aimed specifically at short-term containment of the influence of UV, which is less effective with long-term

exposure to narrow-band light. The anthocyanin protection of the purple-leaved basil is aimed at UV shielding, and the antioxidant system probably is also triggered by UV light.

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