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Analysis of the effects of various light spectra on microgreen species

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ABSTRACT

Light conditions, such as spectral composition, intensity and photoperiod, can affect the photosynthetic apparatus and content of beneficial bioactive compounds. The experiments realised under randomised experimental design tested seedlings of 21 genotypes, including 12 genotypes of the Brassicaceae family, lettuce, amaranth, onion, spinach, fenugreek and two beet and two basil genotypes, which were cultivated in three light environments under narrow-band red and blue light-emitting diodes (LEDs) and broad-band white LEDs. Responsiveness to light spectra was assessed based on fresh and dry weight, total chlorophyll and carotenoid contents and non-invasive assessment of specific compounds using fluorescence excitation ratios. In white light, we observed a higher fresh weight compared to that in monochromatic environments, especially compared to blue light. In most of the species, blue and white light had a positive effect on the concentration of chlorophyll and carotene in plants compared to red light. Blue light was associated with the highest values of chlorophyll excitation ratios, indicating a significant increase in flavonoids and anthocyanin contents. Individual microgreens responded differently in all assessed traits, especially in fluorescence ratios. We also observed the clustering of microgreens with similar responses to LED light spectra. In conclusion, in most plant species, multispectral white LEDs positively influenced quantitative and qualitative traits compared to the most frequently used red LEDs. Blue light slightly improved quality, but suppressed growth. Nevertheless, some species responded differently, emphasising the need to optimise the light to improve the microgreens' production and nutritional value.

Keywords: anthocyanins, flavonoids, fluorescence, light-emitting diodes, pigments, vegetables

INTRODUCTION

One of the most important factors affecting plant growth is light. Plants can absorb approximately 90% of the red and blue light falling through the leaf surface (Terashima et al., 2009), indicating the main signal to which plants respond. It has been extensively demonstrated that different light intensities and photoperiods impact plant metabolism, growth, morphological appearance and nutritional quality of plants (Ward et al., 2005; Liu et al., 2011; Batista et al., 2018). Recently, indoor cultivation using artificial light has been growing, with light-emitting diodes (LEDs) being the most frequent light sources, mainly due to energetic efficiency and the possibility to regulate the light intensity in accordance with the needs of the plants, thereby optimising the production and quality. Cultivation and production of vegetables or fruit crops, edible flowers and microgreens under monochromatic LED lighting can contribute to solving the problems of decreasing arable land due to climate change, population growth, urbanisation and decreasing water availability (Neo et al., 2022; Erekath et al., 2024). Therefore, this kind of plant production is also denoted as vertical agriculture. The aim is to optimise land use and increase production, while reducing the environmental footprint. The system

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offers many advantages, such as a clean source of fresh food throughout the year close to consumers, biological safety, the absence of pests and a large labour market (Haddaji et al., 2023).

Light intensity primarily influences the photosynthetic production (Ghorbanzadeh et al., 2020). In order to adapt to changing light conditions, plants have developed sophisticated physiological systems. Chlorophyll fluorescence methods were originally developed to monitor photosynthesis (Thoren et al., 2010; Kalaji et al., 2014; Porcar-Castell et al., 2021). Even minimal environmental changes cause changes in plant photosynthetic performance and it can be reflected in the fluorescence dynamics. Therefore, fluorescence indicators are often used to determine not only plant stress (Proctor, 2003), but also the level of light and photosynthetic adaptation (Chen et al., 2022). In addition to parameters associated with photosynthetic functions, Cerovic et al. (2002) also introduced the fluorescence excitation ratios (FERs), which can be used to estimate UV-absorbing compounds and anthocyanins in aboveground parts of plants. The development of affordable instrumentation for proximal sensing enabled rapid measurement of chlorophyll fluorescence after excitation with different light spectra simultaneously, thus producing multiple parameters from a single short-term record (Ghozlen et al., 2010). It was shown that the measurements are very sensitive to changes in the contents of phenolic compounds due to changes in the growing light environment (Zivcak et al., 2017; Sytar et al., 2019).

Microgreens are a small form of young (immature), edible vegetables, represented by various herbs, crop seedlings and edible flowers. Microgreens are harvested after 7–21 days, and the size of harvested microgreens is different, depending on the plant species (Lekshmi and Nair, 2023). Microgreens can serve as a rich source of vitamins and minerals and present in higher amounts compared to the mature parts (Bhaswant et al., 2023). The content of bioactive compounds is especially important, as phenolic compounds play the dominant role (Koley and Pandey, 2023). Interestingly, the contents of anti-nutritional factors such as nitrites and nitrates are low in microgreens. Microgreens can be considered a functional food because of the contents of compounds supporting their positive effects on human health (Kyriacou et al., 2019). They can be grown throughout the year because producing them in artificial conditions is easy. Pigments in microgreens, such as anthocyanins and carotenoids, contribute to variability and attractiveness to consumers. In plants from the Amaranthaceae and Chenopodiaceae families, the colour is determined mostly by betalains, giving them the vivid red–pink to yellow colours (Koley and Pandey, 2023).

The fact that the production of microgreens is concentrated in artificial conditions and LED-light illuminated systems provides an opportunity to improve cultivation by manipulating environmental factors, including light. It is well known that different light spectra are active in signalling and regulating not only the growth, but also various metabolic cascades, thus significantly influencing the biosynthesis of various metabolites, including a broad spectrum of bioactive compounds (Landi et al., 2020). Thus, optimising the light quality through selecting proper LEDs may be an efficient tool for improving the production and quality of microgreens. While some partial effects of the light spectra have already been reported, there is limited information on the diversity of responses between different botanical species and genotypes. Therefore, the aim of our study was to study the effects of monochromatic red and blue light, and multichromatic white light emitted by LED-light sources, developed for plant cultivation, in terms of their growth and pigment composition, including the non-invasive assessment of anthocyanin and flavonoid contents. Using a broad collection of species and genotypes grown under the same conditions, the study has ambitions to uncover the specifics and differences in spectral requirements among microgreens.

MATERIALS AND METHODS

Plant material and growing conditions

The experiment was held in a growth chamber at IPES SUA Nitra (Slovakia) from February 2023 to June 2023. The environmental conditions in a growth chamber were precisely regulated and recorded, with a temperature of 23°C/20°C (day/night) and relative air humidity between 60% and 80%. The growth chamber was divided into three separate parts, each with independent illumination provided by the LED arrays: polychromatic warm-white LEDs, monochromatic red LEDs (peak at 660 nm) and monochromatic blue LEDs (peak at 470 nm), as demonstrated in Figure 1.

The lighting system was specifically developed for the cultivation of plants by Photon System Instruments

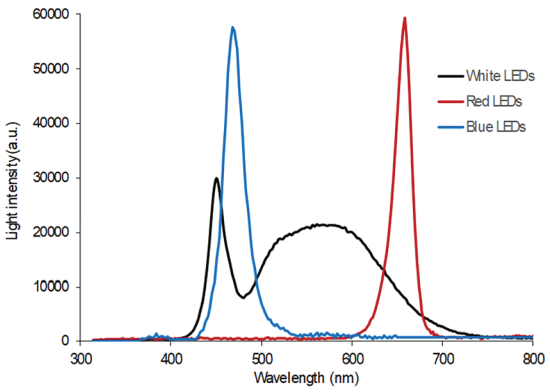


Figure 1. The spectral characteristics of three LED illumination systems applied in the study (measured spectrophotometer SpectraPen mini, Photon Systems Instruments, Czech Republic). LEDs, light-emitting diodes.

(Custom LED Bars Systems by PSI, Czech Republic) and operated automatically. The photoperiod was 14/10 hr (day/night). The light intensity in all three light compartments was set to have similar energy output (~60 W · m^{-2}, which represents PPFD of app. ~160 µmol photons · m^{-2} for blue light, ~175 µmol photons · m^{-2} for white light and up to ~200 µmol photons · m^{-2} for red light) Two subsequent experiments were conducted with identical environmental conditions. The plant material were vegetables and crops cultivated as seedlings (microgreens), belonging to different species and genotypes: amaranth (*Amaranthus tricolour* L.), cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), kohlrabi (*B. oleracea* var. *gongylodes*), arugula (*Eruca vesicaria* subsp. *sativa* (Mill.) Thell.), lettuce (*Lactuca sativa* var. *crispa* L.), cress (*Lepidium sativum* L.), radish 'Red Rambo' and 'Rose' (*Raphanus raphanistrum* subsp. *sativus* (L.), mustard (*Leucosinapis alba* L.), spinach (*Spinacia oleracea* L.), fenugreek (*Trigonella foenum-graecum* L.), pak choi (*Brassica rapa* subsp. *chinensis* L.), mizuna (*B. rapa* subsp. *nipponosica* [L.H.Bailey]), komatsuna (*B. rapa* var. *perviridis*), kale (*B. oleracea* var. *sabellica*), broccoli (*B. oleracea* var. *italica*), beet red (*Beta vulgaris* var. *conditica*), beet yellow (*B. vulgaris* subsp. *vulgaris* 'Yellow'), basil (*Ocimum basilicum* L.), genotypes 'Italian' and 'Red Opal', and onion (*Allium cepa* var. *cepa*). Five microgreens were distinguished as red-leafed (amaranth, radish 'Red Rambo', beet red, mizuna, basil red); the rest were dominantly green-leafed. Plants were cultivated individually in 0.5 L plastic pots in peat substrate in a controlled environment of a growth chamber. Each plant genotype had four pots as replicates under each light (blue, red and white). After 7 days of planting, the seedlings of microgreens typically having epicotyl (stem), cotyledons and the first true leaves in dicots or first two leaves in monocots were measured three times a week with multispectral fluorimeter Multiplex® 3 (Force-A, France) (according to Cerovic et al., 2002), and at the end of the measuring period, the fresh weight (FW), dry weight (DW) and pigment content were analyzed. The experiment was carried out in a randomised design, with four biological replicates (pots) for each genotype.

Fresh and dry weight

At the end of each growing cycle, samples of individual plants were taken, and the fresh weight was immediately measured. After that, the samples were placed in paper bags and dried in an oven at a temperature of 75°C for 2 days until the weight was constant, after which the DM was assessed.

Pigment determination

Samples were taken from each pot with microgreens and weighed, packed into bags, frozen and immediately placed in a deep freezer (−81°C). The samples were extracted by 100% acetone in the presence of $MgCO_3$ and homogenised. Then, the homogenate was diluted with 80% acetone, poured into tubes with a conical bottom, and centrifuged. The absorances were measured spectrophotometrically using a Jenway 6405 UV/VIS (ColeParmer, UK) spectrophotometer, and the chlorophyll and carotenoid contents were calculated using the formulae of Lichtenthaler (1987).

Parameters measured with a multispectral fluorimeter

The multi-excitation and multi-channel measurements of chlorophyll fluorescence were realised by the fluorimeter Multiplex® 3, (Force-A, France). The measurements were realized without contact with plants, from the top, in the central part of the pot, where app. 10 cm^2 was recorded within each flash. Three FERs were calculated: $FER_{R/UV} = F_R/F_{UV}$, where F_R represents the fluorescence intensity measured after red excitation and F_{UV} represents the fluorescence intensity after UV excitation. This parameter represents an indirect estimation of flavonoid content (Cerovic et al., 2002). Second, $FER_{R/G} = F_R/F_G$, where F_R represents the fluorescence intensity measured after red excitation, and F_G represents fluorescence intensity after green excitation. The parameter represents an estimate of anthocyanin content (Ghozlen et al., 2010). The third parameter was Single Fluorescence Ratio, SFR; $SFR = F_{735}/F_{635}$, where F_{735} represents the fluorescence intensity measured after blue excitation in the FR zone, while F_{685} represents the fluorescence intensity after blue excitation in the red zone of the fluorescence spectra. The parameter is usually used as an estimate of surface chlorophyll concentration.

Statistical analysis

The data were processed and analyzed using the software Statistics 10 (Statsoft, Tulsa, OK, USA) and Microsoft Excel. The statistical significance of the effects was analyzed using two-factor analysis of variance (ANOVA), with factors 'light environment' and 'genotype,' followed by a *post hoc* Duncan test (at a significance level of $p < 0.05$) to assess the differences between the treatments. The results are presented as a mean ± standard error. The indicators of the significance of differences are not presented in the article but were used to interpret the results correctly. The grouping of the genotypes was done using hierarchical (cluster) analysis using Statistics 10 (Statsoft) software.

RESULTS AND DISCUSSION

It is well known that light quantity and quality are important factors influencing microgreens' growth and biochemical composition (Bian et al., 2014). In our experiments, we cultivated plants under the same energetic light input, but with different spectral bands. The summary of the results, comparing the effects of different light spectra on the values of parameters for the whole tested collection (Figure 2), indicates not only a large genotypic variability, but also significant effects of light spectra on some, but not all, parameters.

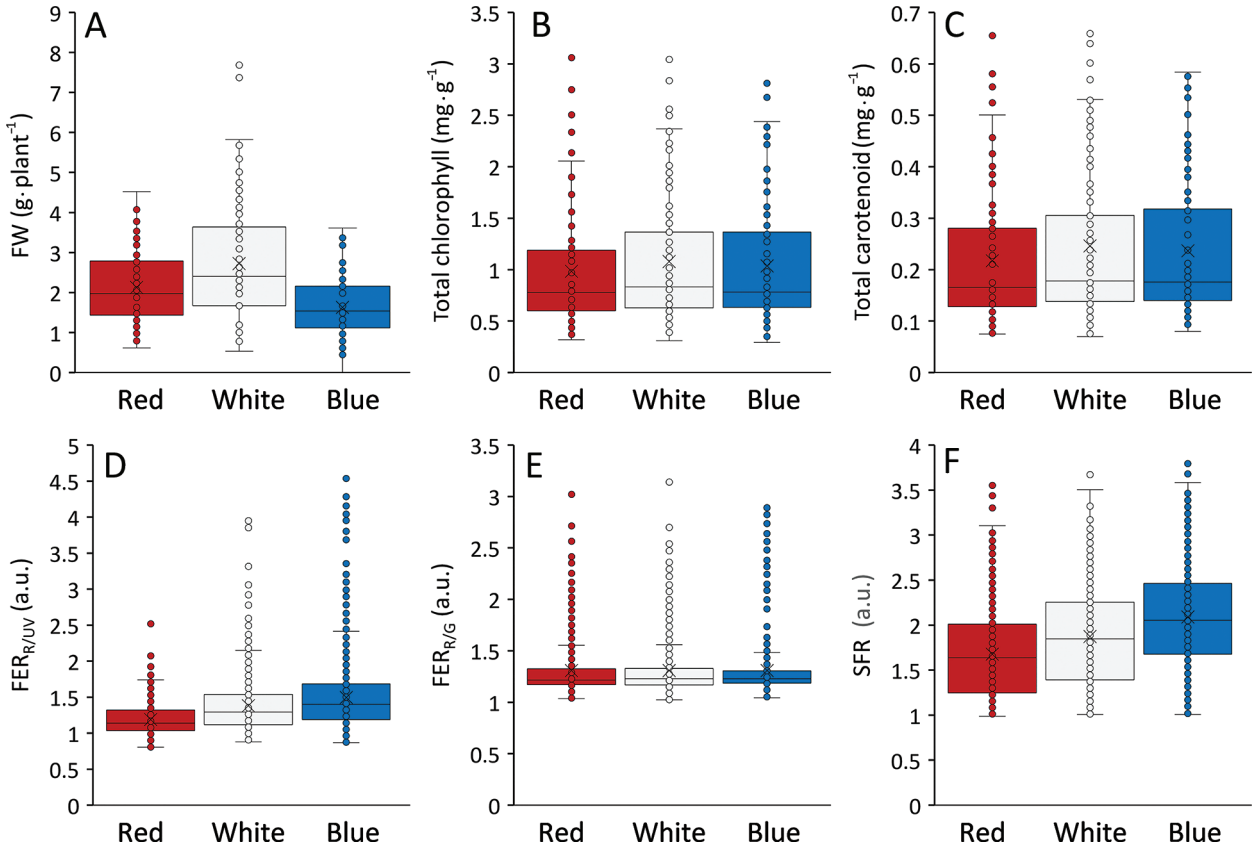


Figure 2. Effect of applied light spectra on the values of the parameters assessed in the experiment in all species and genotypes examined: (A) fresh weight (FW) of individual plants; (B) total chlorophyll content in leaves; (C) total carotenoid contents in leaves; (D) fluorescence excitation ratio R/UV ($FER_{R/UV}$) related to flavonoid content; (E) fluorescence excitation ratio R/G ($FER_{R/G}$) related to anthocyanin content; (F) simple fluorescence ratio (SFR). The point (x) represents the mean value, the line represents the median and the box margins correspond to quartiles. The points show the overall distribution of the values.

In the case of growth, the fresh mass average values point to the highest growth in plants grown in white light, followed by red light. In blue light, the growth was slower. While red light is used most efficiently in photosynthesis, the opening of stomata and transpiration is up-regulated by blue light, which is also essential for the prevention of 'red light syndrome' (Davis and Burns, 2016). Adding blue to red light has been shown to improve plant growth and photosynthesis (Brown et al., 1995; Yorio et al., 2001). The white light contains both red and blue light fractions; so, we can hypothesise that it supports photosynthesis better than each of the monochromatic lights alone. The observed effects in our experiments are in line with previous observations. For example, a positive effect of red light on the growth of broccoli and radish sprouts was noted by Kwach et al. (2015). Lettuce plant biomass was lower under blue light compared to red (Yanagi et al., 1996; Chen et al., 2014). Dou et al. (2017) found that increasing the percentage of blue light over time had a negative effect on both yield and growth.

The effects on chlorophyll (Figure 2B) were less dominant compared to growth, but we still observed an increase of chlorophyll and carotenoid in white and blue light variants compared to red light. Blue light enhances the expression of genes involved in chlorophyll synthesis, while red light at high fluence reduces concentrations of the precursors required for chlorophyll synthesis (Landi et al., 2020). For example, in Chinese cabbage, the presence of blue spectra in light significantly increased chlorophyll concentrations compared to monochromatic red light (Fan et al., 2013). Moreover, it was shown that there are also specific effects on chlorophyll distribution. For example, red light caused hypocotyl etiolation in lettuce, while the addition of blue light prevented this (Hoenecke et al., 1992). As we analysed the chlorophyll contents in all the aboveground parts of the seedling, we found that the specific effects of light spectra on hypocotyl are possible and might influence the values of chlorophyll in our samples.

The general trends observed in our study for chlorophyll and carotenoid contents (Figures 2B vs. 2C) were very similar, with blue light having positive effects on the contents of both groups of pigments. Fu et al. (2012) also found that adding blue light stimulates the synthesis of carotenoids. Alternatively, the pigment contents may also be influenced by the diluting effect due to enhanced plant growth, where biomass grows

faster than pigment biosynthesis, leading to a decrease in pigment concentration per unit of biomass (Lefsrud et al., 2006; Appolloni et al., 2021). The growth was higher in white and red light compared to blue light, providing conditions for a diluting effect compared to slow-growing plants under blue light. Numerous studies report the positive effects of blue light on carotenoid content. For example, it was reported that the application of blue light promoted the accumulation of carotenoids in lettuce (Stutte et al., 2009), while these effects did not appear in spinach (Ohaski-Kaneko et al., 2007). Lobiuc et al. (2017) found that the total carotene content of the *O. basilicum* was not affected by different ratios of red to blue compared to white light. Light quality is not the only parameter that affects the total carotene content. Craver et al. (2017) found that carotene concentrations in microgreens *B. rapa* var *Japonica* and *Brassica juncea* depended on light intensity.

Numerous studies dealing with the cultivation of vegetables under artificial light have recently focused on the production of bioactive compounds, such as phenols (anthocyanins and flavonoids), glutathione and ascorbate, which contribute to the adaptation and acclimation of plants to variable growing conditions (Thoma et al., 2020). Our results confirm the significant effects of light quality on anthocyanin, and especially the flavonoid contents, as indicated by FER values (Figures 2D and 2E). Our general analysis confirmed the positive effect of blue light on flavonoid content, as indicated by the $FER_{R/UV}$ parameter, which was the lowest in red light-grown plants compared to those grown under white light, which contains the fraction of the blue spectra (Figure 1), but the highest values were found in conditions of the monochromatic blue LED light. Previous studies also found a significant increase in total phenolic content under blue LED lighting compared to white in Chinese cabbage and buckwheat sprouts (Qian et al., 2016, Nam et al., 2017). Similarly, increasing the dose of blue light increased the levels of phenolic substances in lamb lettuce (Długosz-Grochowska et al., 2016).

The flavonoids, which are the members of polyphenol group, are characterised by a high phenotypic plasticity in response to light intensity and quality (Landi et al., 2020). They play important physiological roles in plants, especially as sunscreen and antioxidants (Di Ferdinando et al., 2014). The biosynthesis of polyphenols through phenylpropanoid metabolism is well known to be the most efficiently stimulated by the blue light, while the stimulatory effects of other light colours are much less efficient (Huché-Thélier et al., 2016). That fact corresponding to higher values of $FER_{R/UV}$ indicated flavonoid increase as a general trend observed in this study.

Interestingly, the SFR index (Figure 2F) linked to the chlorophyll concentration in the leaves responded more to light spectra than the chlorophyll content itself. The reason might be that the fluorimeter mostly captures information about the top layer cells (Koizumi et al., 1998), which might be slightly different from the results of the wet analysis assessing the full leaf profile.

It is evident that there is a vast genotypic variability in all observed parameters, and the main purpose of our study was to compare genotype effects. The complete list of mean values is shown in Table 1.

The growth was predominantly the highest in white light conditions, as evident from the average data. However, there was also a group of genotypes with opposite response (Table 1). For example, red basil, mizuna and kale grew better under blue light conditions than in white light. In turn, mustard and onion preferred growth under red light. Interestingly, the basil genotypes and mizuna showed higher chlorophyll contents in red light than in blue light, which contradicts most of the genotypes, in which the chlorophyll content is lower in red light conditions compared to white or blue light. It suggests a specific response of basil and mizuna plants to light spectra, which is different from other species in the tested group.

The growth parameters (FW and DW) were significantly influenced by the spectral properties of actinic light, except for onion, in which the changes were insignificant. In seven species and genotypes (Amaranth, kohlrabi, radish, cress, two beets and broccoli), we observed significantly higher growth in white light than in red and blue light. Conditions of red light were most favourable for the growth of mustard, and blue light specifically supported the growth of red basil, kale and mizuna. On the other hand, a decrease of growth in blue light compared to white and red light was observed in numerous species (amaranth, mustard, kohlrabi, cabbage, radish, arugula, fenugreek, spinach, cress and pak choi). The blue light-induced decrease in biomass production can be associated with blue light-induced feedback regulation of photosynthesis (Petroutsos et al., 2016). The blue-light-induced decrease in photosynthesis was observed in numerous species (Landi et al., 2020). On the other hand, there are also studies supporting the trend of enhanced photosynthesis in conditions of blue light; for example, in basil (Lobiuc et al., 2017), which is in accordance with our observation of increased (red basil) or not significantly reduced (green basil) growth of this species in conditions of monochromatic blue irradiation. Interestingly, unlike the increase in biomass, the chlorophyll content was decreased in basil plants in blue light conditions. That partly supports the idea of the diluting effect of intensive biomass growth, which was discussed above. Also, in the case of pigment contents, we observed species with the highest values in white (e.g., Amaranth and mustard), but, in general, the significance of the differences was much lower compared to growth or some other measured parameters.

The effects of the light spectra on values of FER were relatively high. To recognize differences, we plotted the differences between white and red, and blue

Table 1. Values (mean values ± standard error) of the fresh weight (FW), dry weight (DW), fluorescence ratios ($FER_{R/UV}$, $FER_{R/G}$, SFR) and pigment contents in individually tested genotypes*.

Genotype	Variant	FW ($g \cdot plant^{-1}$)	DW ($g \cdot plant^{-1}$)	$FER_{R/UV}$ ($g \cdot plant^{-1}$)	$FER_{R/G}$ (a.u.)	SFR (a.u.)	Total chlorophyll ($mg \cdot g^{-1}$)	Total carotenoids ($mg \cdot g^{-1}$)
Amaranth	Blue	0.510 ± 0.020 a	0.033 ± 0.001 a	1.080 ± 0.000 c	2.480 ± 0.040 c	3.160 ± 0.040 a	1.150 ± 0.070 a	0.275 ± 0.018 a
	Red	1.450 ± 0.060 b	0.083 ± 0.006 b	0.960 ± 0.010 a	1.950 ± 0.020 a	2.180 ± 0.030 a	1.340 ± 0.090 a	0.293 ± 0.013 a
	White	2.440 ± 0.080 c	0.156 ± 0.007 c	1.020 ± 0.010 b	2.140 ± 0.040 b	2.460 ± 0.040 b	1.810 ± 0.100 b	0.488 ± 0.020 b
Mustard	Blue	0.940 ± 0.190 a	0.055 ± 0.011 a	1.430 ± 0.020 c	1.230 ± 0.000 b	1.920 ± 0.070 c	0.600 ± 0.060 b	0.136 ± 0.009 b
	Red	2.350 ± 0.350 b	0.120 ± 0.017 b	1.080 ± 0.010 a	1.170 ± 0.010 a	1.150 ± 0.020 a	0.450 ± 0.030 a	0.098 ± 0.008 a
	White	1.880 ± 0.220 b	0.118 ± 0.019 b	1.350 ± 0.020 b	1.230 ± 0.010 b	1.450 ± 0.040 b	0.750 ± 0.050 c	0.172 ± 0.004 c
Kohlrabi	Blue	1.860 ± 0.310 a	0.107 ± 0.018 a	1.320 ± 0.030 c	1.220 ± 0.010 b	1.760 ± 0.060 b	0.700 ± 0.030 a	0.160 ± 0.005 b
	Red	2.510 ± 0.200 b	0.137 ± 0.004 a	1.110 ± 0.010 a	1.200 ± 0.01 ab	1.300 ± 0.030 a	0.630 ± 0.030 a	0.129 ± 0.011 a
	White	3.090 ± 0.210 c	0.210 ± 0.016 b	1.210 ± 0.020 b	1.160 ± 0.010 a	1.380 ± 0.050 a	0.770 ± 0.050 b	0.169 ± 0.007 b
Cabbage	Blue	1.130 ± 0.380 a	0.060 ± 0.020 a	1.370 ± 0.030 c	1.230 ± 0.000 b	1.800 ± 0.070 b	0.800 ± 0.060 b	0.177 ± 0.012 b
	Red	2.360 ± 0.160 b	0.104 ± 0.009 b	1.140 ± 0.010 a	1.170 ± 0.010 a	1.280 ± 0.020 a	0.610 ± 0.040 a	0.135 ± 0.010 a
	White	3.130 ± 0.410 c	0.180 ± 0.017 c	1.230 ± 0.010 b	1.150 ± 0.010 a	1.360 ± 0.040 a	0.850 ± 0.040 b	0.189 ± 0.007 b
Radish 'Red Rambo'	Blue	2.430 ± 0.290 a	0.116 ± 0.018 a	1.360 ± 0.020 c	1.920 ± 0.040 a	2.050 ± 0.060 c	0.520 ± 0.010 b	0.119 ± 0.002 b
	Red	3.340 ± 0.560 b	0.153 ± 0.028 a	1.150 ± 0.020 a	1.980 ± 0.040 a	1.610 ± 0.030 a	0.440 ± 0.020 a	0.100 ± 0.006 a
	White	5.330 ± 0.410 c	0.261 ± 0.029 b	1.270 ± 0.020 b	1.850 ± 0.050 a	1.740 ± 0.040 b	0.510 ± 0.03 ab	0.119 ± 0.007 b
Radish 'Rose'	Blue	2.670 ± 0.230 a	0.124 ± 0.010 a	1.150 ± 0.020 b	1.180 ± 0.010 a	1.860 ± 0.070 c	0.780 ± 0.080 c	0.161 ± 0.016 b
	Red	3.460 ± 0.190 b	0.162 ± 0.008 a	1.020 ± 0.010 a	1.210 ± 0.010 a	1.360 ± 0.030 a	0.430 ± 0.050 a	0.111 ± 0.010 a
	White	5.680 ± 0.470 c	0.333 ± 0.029 b	1.040 ± 0.010 a	1.180 ± 0.010 a	1.480 ± 0.050 b	0.570 ± 0.040 b	0.127 ± 0.017 a
Arugula	Blue	1.160 ± 0.390 a	0.068 ± 0.024 a	1.330 ± 0.020 c	1.180 ± 0.000 b	1.530 ± 0.030 b	0.770 ± 0.05 ab	0.185 ± 0.006 a
	Red	3.100 ± 0.190 b	0.162 ± 0.010 b	1.100 ± 0.010 a	1.140 ± 0.000 a	1.200 ± 0.020 a	0.730 ± 0.050 a	0.174 ± 0.014 a
	White	3.510 ± 0.390 b	0.241 ± 0.010 c	1.260 ± 0.020 b	1.110 ± 0.010 a	1.220 ± 0.020 a	0.870 ± 0.020 b	0.189 ± 0.009 a
Fenugreek	Blue	0.880 ± 0.070 a	0.068 ± 0.006 a	1.730 ± 0.030 c	1.210 ± 0.010 b	2.540 ± 0.060 c	0.870 ± 0.03 a	0.203 ± 0.004 a
	Red	1.440 ± 0.080 b	0.102 ± 0.003 b	1.310 ± 0.010 a	1.170 ± 0.000 a	2.000 ± 0.040 a	1.030 ± 0.11 ab	0.238 ± 0.020b
	White	1.460 ± 0.210 b	0.123 ± 0.022 c	1.530 ± 0.020 b	1.190 ± 0.01 ab	2.340 ± 0.040 b	1.020 ± 0.080 b	0.232 ± 0.02 ab
Lettuce	Blue	1.750 ± 0.590 a	0.071 ± 0.024 a	0.930 ± 0.010 b	1.150 ± 0.010 a	1.380 ± 0.070 a	1.260 ± 0.080 a	0.309 ± 0.020 b
	Red	2.210 ± 0.490 a	0.096 ± 0.029 a	0.850 ± 0.010 a	1.170 ± 0.010 a	1.320 ± 0.030 a	1.160 ± 0.100 a	0.265 ± 0.015 a
	White	4.380 ± 0.220 b	0.194 ± 0.007 b	0.960 ± 0.000 a	1.140 ± 0.010 a	1.380 ± 0.060 a	1.270 ± 0.040 a	0.305 ± 0.011 b
Spinach	Blue	2.210 ± 0.230 a	0.106 ± 0.008 a	1.470 ± 0.030 b	1.190 ± 0.00 ab	1.800 ± 0.070 b	0.600 ± 0.040 a	0.153 ± 0.010 a
	Red	3.200 ± 0.100 b	0.174 ± 0.010 b	1.170 ± 0.030 a	1.210 ± 0.010 b	1.580 ± 0.030 a	0.970 ± 0.060 b	0.269 ± 0.012 b
	White	3.410 ± 0.450 b	0.223 ± 0.023 b	1.210 ± 0.030 a	1.180 ± 0.010 a	1.700 ± 0.040 a	1.000 ± 0.050 b	0.266 ± 0.015 b
Cress	Blue	0.660 ± 0.260 a	0.033 ± 0.013 a	1.300 ± 0.030 c	1.190 ± 0.000 b	1.900 ± 0.050 c	0.690 ± 0.040 b	0.143 ± 0.001 a
	Red	1.830 ± 0.150 b	0.081 ± 0.005 b	1.050 ± 0.010 a	1.170 ± 0.000 a	1.370 ± 0.030 a	0.530 ± 0.050 a	0.125 ± 0.010 a
	White	3.000 ± 0.480 c	0.182 ± 0.020 c	1.110 ± 0.010 b	1.170 ± 0.01 ab	1.620 ± 0.050 b	0.620 ± 0.02 ab	0.132 ± 0.001 a

(*Continued*)

Table 1. Continued.

Genotype	Variant	FW ($g \cdot plant^{-1}$)	DW ($g \cdot plant^{-1}$)	$FER_{R/UV}$ ($g \cdot plant^{-1}$)	$FER_{R/G}$ (a.u.)	SFR (a.u.)	Total chlorophyll ($mg \cdot g^{-1}$)	Total carotenoids ($mg \cdot g^{-1}$)
Beet Red	Blue	1.550 ± 0.210 a	0.070 ± 0.011 a	1.240 ± 0.020 b	1.420 ± 0.010 a	2.340 ± 0.040 c	0.480 ± 0.030 a	0.111 ± 0.008 a
	Red	1.250 ± 0.110 a	0.062 ± 0.008 a	1.060 ± 0.010 a	1.950 ± 0.060 b	1.840 ± 0.020 a	0.620 ± 0.050 b	0.134 ± 0.02 ab
	White	2.460 ± 0.150 b	0.116 ± 0.008 b	1.220 ± 0.020 b	1.820 ± 0.050 b	2.130 ± 0.040 b	0.600 ± 0.060 b	0.138 ± 0.011 b
Beet Yellow	Blue	3.180 ± 0.220 a	0.140 ± 0.013 a	1.270 ± 0.020 b	1.230 ± 0.010 a	2.040 ± 0.030 c	0.390 ± 0.040 a	0.101 ± 0.007 b
	Red	3.140 ± 0.280 a	0.141 ± 0.010 a	1.140 ± 0.020 a	1.230 ± 0.010 a	1.680 ± 0.020 a	0.440 ± 0.030 a	0.101 ± 0.003 b
	White	4.820 ± 0.470 b	0.253 ± 0.029 b	1.290 ± 0.020 b	1.250 ± 0.010 a	1.840 ± 0.040 b	0.390 ± 0.030 a	0.083 ± 0.005 a
Broccoli	Blue	1.930 ± 0.220 a	0.150 ± 0.014 a	1.450 ± 0.030 b	1.170 ± 0.000 a	1.970 ± 0.060 c	0.720 ± 0.040 a	0.160 ± 0.005 a
	Red	1.790 ± 0.130 a	0.129 ± 0.009 a	1.270 ± 0.030 a	1.170 ± 0.000 a	1.550 ± 0.030 a	0.800 ± 0.040 a	0.154 ± 0.006 a
	White	2.140 ± 0.220 b	0.228 ± 0.029 b	1.380 ± 0.030 b	1.160 ± 0.000 a	1.730 ± 0.040 b	0.720 ± 0.040 a	0.160 ± 0.008 a
Kale	Blue	1.510 ± 0.240 b	0.132 ± 0.014 a	1.070 ± 0.010 b	1.190 ± 0.000 b	1.790 ± 0.070 c	0.430 ± 0.080 a	0.11 ± 0.015 ab
	Red	1.310 ± 0.16 ab	0.133 ± 0.024 a	0.990 ± 0.010 a	1.140 ± 0.010 a	1.190 ± 0.020 a	0.590 ± 0.040 b	0.108 ± 0.005 a
	White	1.040 ± 0.070 a	0.125 ± 0.012 a	1.090 ± 0.010 b	1.160 ± 0.000 a	1.320 ± 0.030 b	0.570 ± 0.020 b	0.124 ± 0.003 b
Komatsuna	Blue	1.740 ± 0.160 a	0.141 ± 0.007 a	1.290 ± 0.020 b	1.280 ± 0.010 c	1.520 ± 0.040 c	0.750 ± 0.020 a	0.165 ± 0.006 a
	Red	2.520 ± 0.240 b	0.222 ± 0.022 b	1.070 ± 0.010 a	1.230 ± 0.010 a	1.130 ± 0.010 a	0.840 ± 0.030 b	0.168 ± 0.006 a
	White	2.700 ± 0.340 b	0.267 ± 0.024 b	1.220 ± 0.010 b	1.270 ± 0.010 b	1.310 ± 0.020 b	0.770 ± 0.10 ab	0.162 ± 0.017 a
Mizuna	Blue	1.480 ± 0.200 b	0.105 ± 0.011 a	1.590 ± 0.020 b	1.540 ± 0.010 a	1.780 ± 0.060 b	0.800 ± 0.070 a	0.182 ± 0.016 b
	Red	1.290 ± 0.12 ab	0.109 ± 0.018 a	1.370 ± 0.020 a	1.680 ± 0.020 b	1.310 ± 0.020 a	0.780 ± 0.040 a	0.147 ± 0.006 a
	White	1.110 ± 0.090 a	0.085 ± 0.009 a	1.580 ± 0.020 b	1.650 ± 0.010 b	1.330 ± 0.020 a	0.800 ± 0.060 a	0.168 ± 0.008 b
Pak Choi	Blue	1.300 ± 0.160 a	0.106 ± 0.018 a	1.170 ± 0.010 c	1.200 ± 0.000 c	1.800 ± 0.050 c	0.650 ± 0.11 ab	0.143 ± 0.02 ab
	Red	1.990 ± 0.180 b	0.202 ± 0.021 b	0.990 ± 0.010 a	1.150 ± 0.010 a	1.130 ± 0.020 a	0.570 ± 0.080 a	0.114 ± 0.016 a
	White	2.000 ± 0.190 b	0.221 ± 0.024 b	1.100 ± 0.010 b	1.180 ± 0.000 b	1.360 ± 0.030 b	0.750 ± 0.080 b	0.165 ± 0.008 b
Basil 'Italian'	Blue	2.000 ± 0.180 a	0.122 ± 0.012 a	1.230 ± 0.020 c	1.150 ± 0.010 b	1.330 ± 0.040 b	0.550 ± 0.040 a	0.125 ± 0.005 a
	Red	2.110 ± 0.150 a	0.145 ± 0.013 a	1.010 ± 0.000 a	1.080 ± 0.010 a	1.090 ± 0.020 a	0.750 ± 0.090 b	0.154 ± 0.008 b
	White	1.870 ± 0.070 a	0.117 ± 0.007 a	1.130 ± 0.010 b	1.110 ± 0.000 a	1.320 ± 0.070 b	0.700 ± 0.070 b	0.131 ± 0.01 ab
Basil 'Red'	Blue	1.370 ± 0.050 b	0.067 ± 0.002 b	0.990 ± 0.000 b	1.510 ± 0.010 b	1.630 ± 0.050 b	0.650 ± 0.070 a	0.139 ± 0.008 a
	Red	1.180 ± 0.14 ab	0.054 ± 0.004 a	0.950 ± 0.000 a	1.440 ± 0.010 a	1.410 ± 0.040 a	0.830 ± 0.030 b	0.180 ± 0.003 b
	White	1.020 ± 0.070 a	0.051 ± 0.003 a	0.990 ± 0.010 b	1.510 ± 0.010 b	1.640 ± 0.060 b	0.730 ± 0.07 ab	0.170 ± 0.012 ab
Onion	Blue	0.690 ± 0.060 a	0.050 ± 0.003 a	1.100 ± 0.020 a	1.150 ± 0.010 a	2.070 ± 0.070 b	0.610 ± 0.090 a	0.142 ± 0.017 a
	Red	0.790 ± 0.060 a	0.053 ± 0.004 a	1.070 ± 0.010 a	1.150 ± 0.000 a	1.830 ± 0.040 a	0.680 ± 0.040 a	0.150 ± 0.01 2 a
	White	0.750 ± 0.080 a	0.053 ± 0.002 a	1.110 ± 0.020 a	1.150 ± 0.010 a	1.960 ± 0.040 b	0.640 ± 0.040 a	0.141 ± 0.013 a

*Values with different lowercase letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

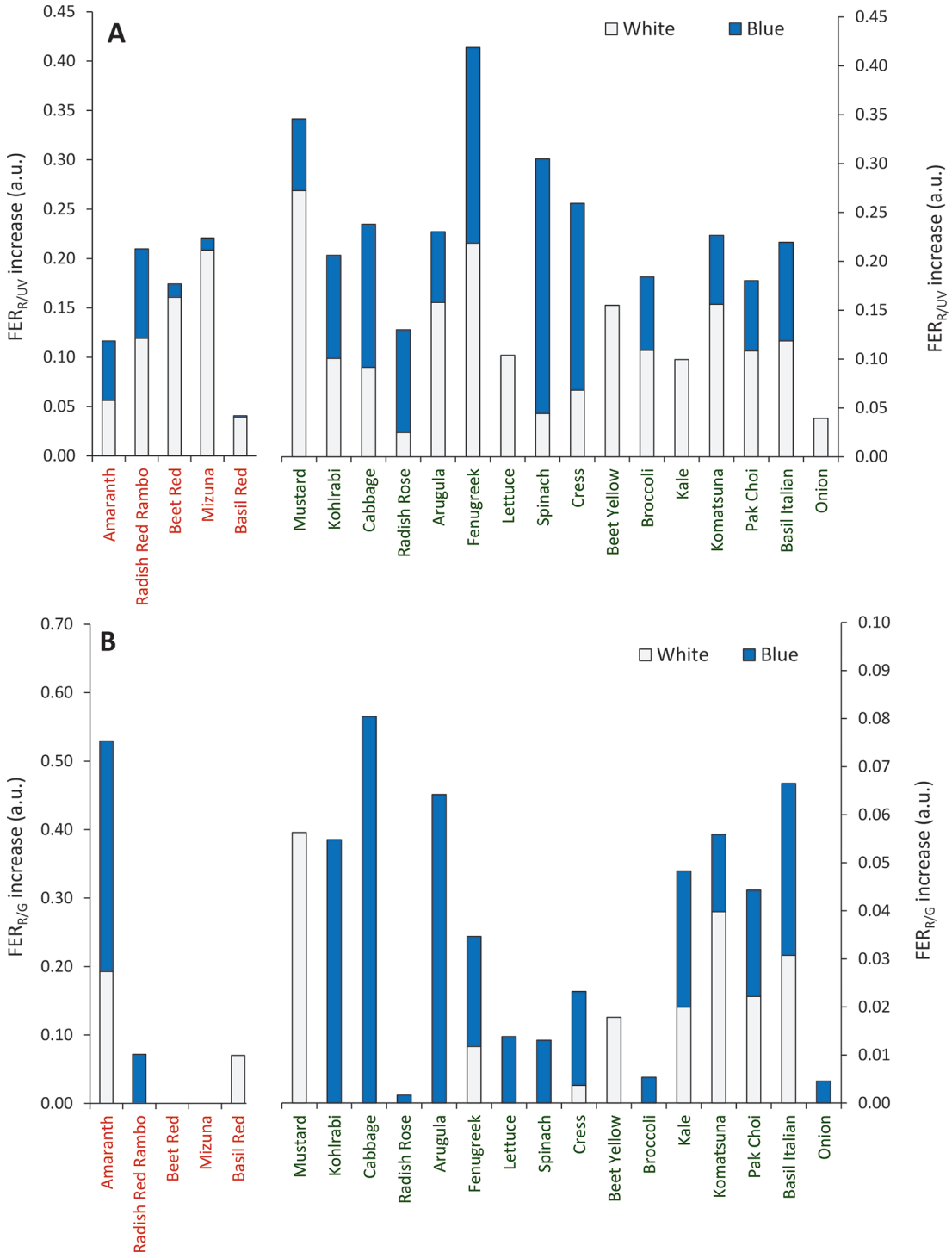


Figure 3. Increase of fluorescence excitation indexes indicating (A) flavonoid content (FER$_{R/UV}$) and (B) anthocyanin content (FERR/G) in plants exposed to white and blue light compared to red light LEDs. Five red-leafed genotypes are presented separately (left graph) from the green-leafed genotypes (graph red). In the case of anthocyanin index (B), the changes in red (graphs left) and green leafed (graphs right) genotypes are presented in different scales. LEDs, light-emitting diodes.

and white light-grown plants (Figure 3), with a separate presentation of red-leafed and green-leafed genotypes.

In most species, we observed an increase of FER$_{R/UV}$ (flavonoids) in white light-grown plants compared to red light-grown plants, and additional increase as a response to blue light, thus exceeding the values observed in plants grown under white light (Figure 3A). However, there were some exceptions, such as low gain of flavonoids in white light compared to red light in spinach, red basil and rose radish. Moreover, in red basil, lettuce, kale and onion, we did not observe a significant difference between white-exposed and blue-

exposed plants. It indicates that in a smaller group of species (e.g., lettuce, kale and beets), the contribution of blue light in white light spectra (shown in Figure 1) was sufficient to stimulate the accumulation of flavonoids at a similar level to the monochromatic blue light. On the other hand, most of the species responded to monochromatic blue light with a significantly higher accumulation of flavonoids compared to red and blue light, which corresponds to expected trends (Huché-Thélier et al., 2016; Landi et al., 2020).

The grouping based on leaf colour was, however, not observed.

A similar situation was also found in the case of the parameter of $FER_{R/G}$ (anthocyanins), where we assessed separately the red and green species, as they differed significantly in the scale of the measured signal (Figure 3B). Interestingly, from the red-leafed species, we observed a significant difference between different light spectra in *Amaranth*. In contrast, in the other red-leafed genotypes, we observed a very tiny effect of the light spectra. In green-leafed genotypes, we also observed some increases in anthocyanin contents, mostly induced by blue light. The exception was mustard, in which additional blue light did not lead to more anthocyanin. Moreover, there are also species like radish, onion and broccoli, which do not respond to different light by a higher anthocyanin content. The response of anthocyanin content to blue light is expected and clearly linked to receptors of blue light (Ahmed et al., 1995; Huché-Thélier et al., 2016). The lack of additional blue-light-induced accumulation of anthocyanins, especially in red-leafy species, such as mizuna or red basil, is difficult to explain and will require further examination.

As there are many specific responses in multiple genotypes, we tried to visualise the effects of different spectra using a comparison of parameters in plants of different species and genotypes grown in red, white and blue LED illumination (Figure 3).

The heat map shows that for each parameter, some genotypes respond differently compared to the average trend. It indicates differences between the species and genotypes in preference of light spectra. The heat map also indicates some genotypes with similar patterns (e.g., mustard and pak choi), but there are also very unequal responses.

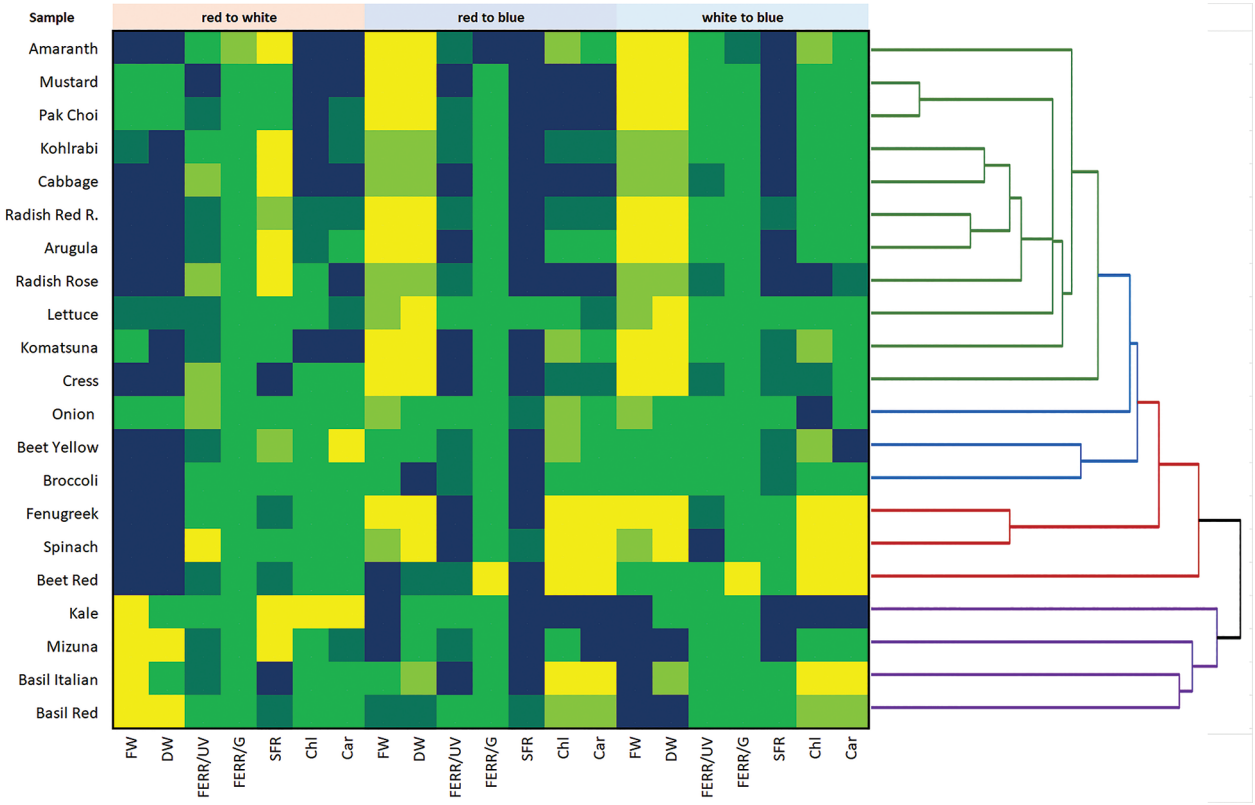


Figure 4. Heat map representing the changes in parameters in mutual comparison ('red compared to white', 'red compared to blue' or 'white compared to blue') of parameters in plants of different species and genotypes grown in red, white and blue LED illumination. Dark blue colour represents a positive difference of more than 20%, dark green of 10%–20%; pale green colour represents similar values (difference less than 10%); yellow–green colour represents a negative difference (decrease) by 10%–20%, and yellow colour indicates a negative difference (decrease) by more than 20% or more for each mutual comparison. The hierarchical clustering of evaluated genotypes is based on relative changes in the values of all parameters assessed in the study. The dendrogram indicates the clusters in which the samples showed similar responses to different LED light spectra. LED, light-emitting diode.

Using the data, we analysed the values using hierarchic analysis (Figure 4). Individual genotypes created the clusters, identifying similar responses to light. For example, two basil genotypes were clustered together, and the identified distance between two radish genotypes was also relatively low. There is a separate branch represented by two genotypes of basil, mizuna and kale, in which we can observe some preference of blue light spectra not only over the red light, but also partly over the blue light. Very specific was also the response of red beet, which responded very well to blue light in terms of growth, but blue did not stimulate a higher accumulation of pigments and bioactive compounds, as was observed in many other species. While in most species, we observed that the negative effect of blue light on growth was balanced by some positive effects on some other parameters, especially the content of bioactive compounds; in the case of lettuce, we observed only minor positive effects. It indicates the preference for the red spectrum of LED lights in this species.

While in most production systems, a high growth rate is required and represents the most important trait, in microgreens, the advantage is not as straightforward, as slower growth dynamics might be associated with a longer harvest time. Thus, by changing the light quality, growers could partly manage delivery period, while gaining a higher content of bioactive compounds. The blue-light cultivation might also represent an advantage if the microgreens are temporarily further cultivated by end-users, where blue spectra might delay overgrowth and, thus, increase the duration of microgreens' harvest.

Considering that both red and blue light affect the growth and synthesis of beneficial compounds in plants by different mechanisms and that the responses are specific for each species/cultivar, adaptation of yield and quality is now a matter of finding optimal ratios of light wavelengths and other growing conditions (Lobiuc et al., 2017). That will require further research, focusing more on microgreens' technological and qualitative attributes.

CONCLUSION

The study clearly demonstrated the significant effects of light spectra on the growth and contents of pigments in plants of microgreens. Moreover, the hypothesis that the diverse species and genotypes respond differently to various light spectra was confirmed. The study enabled the grouping of the species and genotypes according to their responses to light. In general, white LEDs supported growth and had good effects on other assessed parameters in most species, which emphasises the use of broader spectral bands instead of monochromatic LEDs. However, we also identified the species with a higher preference for blue light (e.g., basil genotypes, mizuna and kale) over red light (e.g. mustard and pak choi). The study also confirmed that manipulating light spectra might positively influence the quantitative and qualitative traits in microgreens, as demonstrated, for example, on the contents of flavonoids and anthocyanins. On the other hand, the responses to spectral changes were genotype-specific, and the optimum light environments should be fine-tuned individually for each cultivated species.

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AUTHOR CONTRIBUTIONS

L.J., M.Ba. and J.F. performed the measurements. L.J., M.Ž. and A.F., managed the data. M.Ž. and M.Br. designed the study and directed the projects. M.Br. and M.Z. supervised the projects. L.J., and M.Ž. analysed the data and designed the figures. L.J. and M.Ž. wrote the manuscript with consultations from M.Br.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ahmad, M., Lin, C., and Cashmore, A. R. (1995). Mutations throughout an *Arabidopsis* blue-light photoreceptor impair blue-light-responsive anthocyanin accumulation and inhibition of hypocotyl elongation. *The Plant Journal, 8*(5), 653–658, https://doi.org/10.1046/j.1365-313X.1995.08050653.x.

Appolloni, E., Pennisi, G., Zauli, I., Carotti, L., Paucek, I., Quaini, S., Orsini, F., and Gianquinto, G. (2021). Beyond vegetables: Effects of indoor LED light on specialized metabolite biosynthesis in medicinal and aromatic plants, edible flowers, and microgreens. *Journal of the Science of Food and Agriculture, 102*(2), 472–487, https://doi.org/10.1002/jsfa.11513.

Batista, D., Felipe, S. H. S., Silva, T. D., De Castro, K. M., Mamedes-Rodrigues, T. C., Miranda, N., Ríos, A. M. R., Faria, D., Fortini, E. A., Chagas, K., Torres-Silva, G., Xavier, A., Arencibia, A. D., and Otoni, W. C. (2018). Light quality in plant tissue culture: Does it matter? *In Vitro Cellular & Developmental Biology – Animal, 54,* 195–215.

Bhaswant, M., Shanmugam, D. K., Miyazawa, T., Abe, C., and Miyazawa, T. (2023). Microgreens – A comprehensive review of bioactive molecules and health benefits. *Molecules, 28*(2), 867, https://doi.org/10.3390/molecules28020867.

Bian, Z., Yang, Q., and Liu, W. K. (2014). Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments:

A review. *Journal of the Science of Food and Agriculture, 95*(5), 869–877, https://doi.org/10.1002/jsfa.6789.

Brown, C. S., Schuerger, A. C., and Sager, J. C. (1995). Growth and photomorphogenesis of pepper plants grown under red light-emitting diodes, supplemented with blue or far-red illumination. *Journal of the American Society for Horticultural Science, 120,* 808–813.

Cerovic, Z. G., Ounis, A., Cartelat, A., Latouche, G., Goulas, Y., Meyer, S., and Moya, I. (2002). The use of chlorophyll fluorescence excitation spectra for the non-destructive, *in situ* assessment of UV-absorbing compounds in leaves. *Plant, Cell and Environment, 25*(12), 1663–1676, https://doi.org/10.1046/j.1365-3040.2002.00942.x.

Chen, D., Zhang, J., Zhang, Z., Wan, X., and Jin, H. (2022). Analyzing the effect of light on lettuce Fv/Fm and growth by machine learning. *Scientia Horticulturae, 306,* 111444, https://doi.org/10.1016/j.scienta.2022.111444.

Chen, X. L., Guo, W. Z., Xue, X. Z., Wang, L. C., and Qiao, X. J. (2014). Growth and quality responses of 'Green Oak Leaf' lettuce as affected by monochromic or mixed radiation provided by a fluorescent lamp (FL) and light-emitting diode (LED). *Scientia Horticulturae, 172,* 168–175, https://doi.org/10.1016/j.scienta.2014.04.009.

Craver, J. K., Gerovac, J. R., Lopez, R. G., and Kopsell, D. A. (2017). Light intensity and light quality from sole-source, light-emitting diodes impact phytochemical concentrations within *Brassica* microgreens. *Journal of the American Society for Horticultural Science, 142*(1), 3–12, https://doi.org/10.21273/jashs03830-16.

Davis, P. A., and Burns, C. O. (2016). Photobiology in protected horticulture. *Food and Energy Security, 5*(4), 223–238, https://doi.org/10.1002/fes3.97.

Di Ferdinando, M., Brunetti, C., Agati, G., and Tattini, M. (2014). Multiple functions of polyphenols in plants inhabiting unfavorable Mediterranean areas. *Environmental and Experimental Botany, 103,* 107–116, https://doi.org/10.1016/j.envexpbot.2013.09.012.

Długosz-Grochowska, O., Kołton, A., and Wojciechowska, R. (2016). Modifying folate and polyphenol concentrations in lamb's lettuce by the use of LED supplemental lighting during cultivation in greenhouses. *Journal of Functional Foods, 26,* 228–237, https://doi.org/10.1016/j.jff.2016.07.020.

Dou, H., Niu, G., Gu, M., and Masabni, J. G. (2017). Effects of light quality on growth and phytonutrient accumulation of herbs under controlled environments. *Horticulturae, 3*(2), 36, https://doi.org/10.3390/horticulturae3020036.

Erekath, S., Seidlitz, H., Schreiner, M., and Dreyer, C. (2024). Food for future: Exploring cutting-edge technology and practices in vertical farm. *Sustainable Cities and Society, 106*(1), 105357, http://dx.doi.org/10.1016/j.scs.2024.105357.

Fan, X., Zang, J., Xu, Z., Guo, S., Jiao, X., Li, X., and Gao, Y. (2013). Effects of different light quality on growth, chlorophyll concentration and chlorophyll biosynthesis precursors of non-heading Chinese cabbage (*Brassica campestris* L.). *Acta Physiologiae Plantarum, 35*(9), 2721–2726, https://doi.org/10.1007/s11738-013-1304-z.

Fu, W., Gudmundsson, Ó., Paglia, G., Herjolfsson, G., Andrésson, Ó. S., Palsson, B. O., and Brynjólfsson, S. (2012). Enhancement of carotenoid biosynthesis in the green microalga *Dunaliella salina* with light-emitting diodes and adaptive laboratory evolution. *Applied Microbiology and Biotechnology, 97*(6), 2395–2403, https://doi.org/10.1007/s00253-012-4502-5.

Ghorbanzadeh, P., Aliniaeifard, S., Esmaeili, M., Mashal, M., Azadegan, B., and Seif, M. (2020). Dependency of growth, water use efficiency, chlorophyll fluorescence and stomatal characteristics of lettuce plants to light intensity. *Journal of Plant Growth Regulation, 40*(5), 2191–2207, https://doi.org/10.1007/s00344-020-10269-z.

Ghozlen, N. B., Cerovic, Z. G., Germain, C., Toutain, S., and Latouche, G. (2010). Non-destructive optical monitoring of grape maturation by proximal sensing. *Sensors, 10*(11), 10040–10068, https://doi.org/10.3390/s101110040.

Haddaji, H. E., Akodad, M., Skalli, A., Moumen, A., Bellahcen, S., Elhani, S., Urrestarazu, M., Kolar, M., Imperl, J., Petrova, P., and Baghour, M. (2023). Effects of light-emitting diodes (LEDs) on growth, nitrates and osmoprotectant content in microgreens of aromatic and medicinal plants. *Horticulturae, 9*(4), 494, https://doi.org/10.3390/horticulturae9040494.

Hoenecke, M. E., Bula, R. J., and Tibbitts, T. W. (1992). Importance of blue photon levels for lettuce seedlings, grown under red-light emitting diodes. *HortScience, 27,* 427–430.

Huché-Thélier, L., Crespel, L., Le Gourrierec, J., Morel, P., Sakr, S., and Leduc, N. (2016). Light signalling and plant responses to blue and UV radiations – Perspectives for applications in horticulture. *Environmental and Experimental Botany, 121,* 22–38, https://doi.org/10.1016/j.envexpbot.2015.06.009.

Kalaji, H. M., Schansker, G., Ladle, R. J., Goltsev, V., Bosa, K., Allakhverdiev, S. I., Brestic, M., Bussotti, F., Calatayud, A., Dąbrowski, P., Elsheery, N. I., Ferroni, L., Guidi, L., Hogewoning, S. W., Jajoo, A., Misra, A. N., Nebauer, S. G., Pancaldi, S., Penella, C., Poli, D., Pollastrini, M., Romanowska-Duda, Z. B., Rutkowska, B., Serôdio, J., Suresh, K., Szulc, W., Tambussi, E., Yanniccari, M., and Zivcak, M. (2014). Frequently asked questions about *in*

vivo chlorophyll fluorescence: Practical issues. *Photosynthesis Research, 122,* 121–158, https://doi.org/10.1007/s11120-014-0024-6.

Koizumi, M., Takahashi, K., Mineuchi, K., Nakamura, T., and Kano, H. (1998). Light gradients and the transverse distribution of chlorophyll fluorescence in mangrove and *camellia* leaves. *Annals of Botany, 81*(4), 527–533, https://doi.org/10.1006/anbo.1998.0589.

Koley, T. K., and Pandey, V. (2023). Microgreens from vegetables: More nutrition for better health. In: B. Singh and P. Kalia (Eds), *Vegetables for Nutrition and Entrepreneurship* (pp. 103–113). Singapore: Springer, https://doi.org/10.1007/978-981-19-9016-8_6.

Kwack, Y., Kim, K. K., Hwang, H., and Chun, C. (2015). Growth and quality of sprouts of six vegetables cultivated under different light intensity and quality. *Horticulture, Environment and Biotechnology, 56*(4), 437–443, https://doi.org/10.1007/s13580-015-1044-7.

Kyriacou, M. C., El-Nakhel, C., Graziani, G., Pannico, A., Soteriou, G. A., Giordano, M., and Rouphael, Y. (2019). Functional quality in novel food sources: Genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. *Food Chemistry, 277,* 107–118.

Landi, M., Zivcak, M., Sytar, O., Brestic, M., and Allakhverdiev, S. I. (2020). Plasticity of photosynthetic processes and the accumulation of secondary metabolites in plants in response to monochromatic light environments: A review. *Biochimica et Biophysica Acta – Bioenergetics, 1861*(2), 148131, https://doi.org/10.1016/j.bbabio.2019.148131.

Lefsrud, M., Kopsell, D. A., Kopsell, D. E., and Curran-Celentano, J. (2006). Irradiance levels affect growth parameters and carotenoid pigments in kale and spinach grown in a controlled environment. *Physiologia Plantarum, 127*(4), 624–631, https://doi.org/10.1111/j.1399-3054.2006.00692.x.

Lekshmi, G. P., and Nair, B. R. (2023). Microgreens: A future super food. In: S. T. Sukumaran and T. R. Keerthi (Eds), *Conservation and Sustainable Utilisation of Bioresources* (pp. 103–122). Singapore: Springer, https://doi.org/10.1007/978-981-19-5841-0_5.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic bio membranes. *Methods Enzymology, 148c,* 350–382.

Liu, X., Chang, T., Guo, S., Xu, Z., and Li, J. (2011). Effect of different light quality of lead on growth and photosynthetic character in cherry tomato seedling. *Acta Horticulturae, 907,* 325–330.

Lobiuc, A., Vasilache, V., Pintilie, O., Stoleru, T., Burducea, M., Oroian, M., and Zamfirache, M. (2017). Blue and red LED illumination improves growth and bioactive compounds contents in acyanic and cyanic *Ocimum basilicum* L. microgreens. *Molecules, 22*(12), 2111, https://doi.org/10.3390/molecules22122111.

Nam, T. G., Kim, D., and Eom, S. H. (2017). Effects of light sources on major flavonoids and antioxidant activity in common buckwheat sprouts. *Food Science and Biotechnology, 27*(1), 169–176, https://doi.org/10.1007/s10068-017-0204-1.

Neo, D. C. J., Ong, M. M. X., Lee, Y. Y., Teo, E. J., Ong, Q., Tanoto, H., Xu, J., Ong, K. S., and Suresh, V., (2022). Shaping and tuning lighting conditions in controlled environment agriculture: A review. *ACS Agricultural Science & Technology, 2*(1), 3–16.

Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K., and Kurata, K. (2007). Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. *Environmental Control in Biology, 45*(3), 189–198, https://doi.org/10.2525/ecb.45.189.

Petroutsos, D., Tokutsu, R., Maruyama, S., Flori, S., Greiner, A., Magneschi, L., Cusant, L., Kottke, T., Mittag, M., Hegemann, P., and Finazzi, G. (2016). A blue-light photoreceptor mediates the feedback regulation of photosynthesis. *Nature, 537*(7621), 563–566, https://doi.org/10.1038/nature19358.

Porcar-Castell, A., Malenovský, Z., Magney, T., Van Wittenberghe, S., Fernández-Marín, B., Maignan, F., Zhang, Y., Maseyk, K., Atherton, J., Albert, L. P., and Robson, T. M. (2021). Chlorophyll a fluorescence illuminates a path connecting plant molecular biology to earth-system science. *Nature Plants, 7*(8), 998–1009.

Proctor, M. C. F. (2003). Experiments on the effect of different intensities of desiccation on bryophyte survival, using chlorophyll fluorescence as an index of recovery. *Journal of Bryology, 25*(3), 201–210, https://doi.org/10.1179/037366803235001652.

Qian, H., Li, T., Deng, M., Miao, H., Cai, C., Shen, W., and Wang, Q. (2016). Effects of light quality on main health-promoting compounds and antioxidant capacity of Chinese kale sprouts. *Food Chemistry, 196,* 1232–1238, https://doi.org/10.1016/j.foodchem.2015.10.055.

Stutte, G. W., Edney, S., and Skerritt, T. (2009). Photo regulation of bioprotectant content of red leaf lettuce with light-emitting diodes. *HortScience, 44,* 79–82.

Sytar, O., Zivcak, M., Neugart, S., Toutounchi, P. M., and Brestic, M. (2019). Precultivation of young seedlings under different colour shades modifies the accumulation of phenolic compounds in Cichorium leaves in later growth phases. *Environmental and Experimental Botany, 165,* 30–38, https://doi.org/10.1016/j.envexpbot.2019.05.018.

Terashima, I., Fujita, T., Inoue, T., Chow, W. S., and Oguchi, R. (2009). Green light drives leaf

photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of why leaves are green. *Plant & Cell Physiology, 50,* 684–697.

Thoma, F., Somborn-Schulz, A., Schlehuber, D., Keuter, V., and Deerberg, G. (2020). Effects of light on secondary metabolites in selected leafy greens: A review. *Frontiers in Plant Science, 11,* 497, https://doi.org/10.3389/fpls.2020.00497.

Thoren, D., Thorén, P., and Schmidhalter, U. (2010). Influence of ambient light and temperature on laser-induced chlorophyll fluorescence measurements. *European Journal of Agronomy, 32*(2), 169–176, https://doi.org/10.1016/j.eja.2009.10.003.

Ward, J. M., Cufr, C. A., Denzel, M. A., and Neff, M. M. (2005). The Dof transcription factor OBP3 modulates phytochrome and cryptochrome signalling in *Arabidopsis*. *Plant Cell, 17,* 475–485.

Yanagi, T., Okamoto, K., and Takita, S. (1996). Effects of blue, red, and blue/red lights of two different ppf levels on growth and morphogenesis of lettuce plants. *Acta Horticulturae, 440,* 117–122, https://doi.org/10.17660/actahortic.1996.440.21.

Yorio, N. C., Goins, G. D., Kagie, H. R., Wheeler, R. M., and Sager, J. C. (2001). Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *HortScience, 36,* 380–383.

Zivcak, M., Bruckova, K., Sytar, O., Brestic, M., Olsovska, K., and Allakhverdiev, S. I. (2017). Lettuce flavonoids screening and phenotyping by chlorophyll fluorescence excitation ratio. *Planta, 245*(6), 1215–1229, https://doi.org/10.1007/s00425-017-2676-x.

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