

Light matters: Effect of light spectra on cannabinoid profile and plant development of medical cannabis (*Cannabis sativa* L.)

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ABSTRACT

Light is a key factor affecting plant growth, metabolism and function. Metabolic processes in plants are sensitive to the ratio of Blue:Red light, and there is an increasing awareness that the response to the ratio of these monochromatic lights may vary under exposure to a wider range of the spectrum, such as white light. Due to the potential for regulation of the therapeutic chemical profile and plant development, this issue is of growing interest for the cannabis (*Cannabis sativa* L.) industry that uses photosynthetic light extensively. Cannabis is a medicinal plant treasured for its secondary metabolites, especially cannabinoids, the unique biologically active compounds in the plant that are considered to be affected by light spectra. In this study we evaluated the hypothesis that the ratio of Blue:Red light affects cannabinoid metabolism, and that plant growth and secondary metabolism is intensified under a full spectrum with similar Blue:Red ratio. Our results point to several spectra specific reactions and some cultivar dependent responses to light spectrum. i. Yield quantity: The highest inflorescence yields were obtained when the spectrum was restricted to the red and blue range at the ratio of 1:1, and in two of the three varieties tested a ratio of 1:4 Blue:Red light had similar results. White light with Blue:Red ratio of 1:1 had the lowest yield. ii. The chemical profile was also affected by the light spectrum, and CBGA, the primary cannabinoid and a precursor for most other cannabinoids, demonstrated the highest response. CBGA accumulation was stimulated by blue-rich light as compared with far-red rich HPS light. The major cannabinoids CBDA, THCA and CBCA were also affected by light quality, and the response was cultivar specific and less pronounced than for CBGA. iii. Plant morphology: Blue light was most inductive for maintaining compact plants, more so than Red:Far-Red ratio. Our results refute the hypothesis that full spectrum improves inflorescence yield compared with Blue:Red light, but support the hypothesis that light spectrum influences plant development and the cannabinoid profile, which could be used to fine-tune cannabis and cannabinoid production.

1. Introduction

Light is one of the main signals perceived by plants that affects plant growth, development and function (Kami et al., 2010). Major light features that impact plants are light intensity, light quality (the spectral properties of light) and the photoperiod (the duration of exposure to light) (Bian et al., 2015; Ouzounis et al., 2015). These factors affect plants' function by involvement in the regulation of three main plant processes: photosynthesis, photoperiodism, and photomorphogenesis.

Photosynthetic carbon fixation requires light energy and is responsive mostly to light intensity at specific wavelengths (Evans and Poorter, 2001). The photosynthetic active radiation ranges from 400–700 nm. The most influential wavelengths are conventionally considered to be in

the red (600–700 nm) and blue (420–450 nm) zones of the spectrum, which are the maximum absorption ranges for chlorophylls a and b, with carotenoids adding absorption in the 470–700 nm range. However, recent studies suggested beneficial effects of full spectrum (white light) to plant growth and function, compared to monochromatic red and blue lights (Landi et al., 2020; Smith et al., 2017). Photosynthesis is directly affected by light intensity, with higher photon flux density at the selective wavelengths supporting higher rates of carbon fixation (Feng et al., 2019). 'Drug type' medical cannabis produces unusually large amounts of secondary metabolites, up to 20 % of the dry weight basis is not unusual, which entails immense energy demands. The response of photosynthesis to light spectrum and intensity is therefore important for understanding regulation of energy balance and secondary metabolism

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in the plant.

Photoperiodism, is the developmental responses of plants to the daily lengths of light and dark periods, and is based on the plants ability to sense elapsed time since sunrise or sundown and to regulate processes according to time of day and day length (Jackson, 2009). Photoperiodic responses include also flowering initiation (Song et al., 2015), which is of high importance for the development progression in a short-day plant such as *Cannabis sativa*.

Photomorphogenesis, is the effect of light quality, or spectrum, on plant development and physiology. It affects major plant developmental stages including the switch from the vegetative to the flowering stage, and involves in elongation (Reed et al., 1993), stomatal conductance (Kim et al., 2004), leaf expansion (Kong and Zheng, 2020), as well as secondary metabolism (Ouzounis et al., 2015). It is also affected by photoperiodism (Nagy and Schäfer, 2002). Unlike photosynthesis which utilizes light as a source of energy, both photomorphogenesis and photoperiodism are triggered by activation of pigments such as phototropins, cryptochromes and phytochromes by specific wavelengths even at low levels of irradiance (Boccalandro et al., 2012; Huché-Thélier et al., 2016; Nagy and Schäfer, 2002). Light quality is therefore expected to critically affect physiology, developmental progression, morpho-development and secondary metabolism in *Cannabis sativa*, our model plant of study.

Cannabis, which is recognized by humanity for thousands of years for its medical, recreational and industrial potential, is having a boost of global recognition and cultivation due to recent changes in regulations, that also facilitate plant-science research and development. The medical properties of cannabis stem from the plethora of secondary metabolites such as cannabinoids, flavonoids and terpenes, over a thousand of which were identified and described in the plant inflorescence (Andre et al., 2016). Variations in quantities and ratios between these metabolites may affect the therapeutic potential and patients' response (Russo, 2019). Secondary metabolism in plants is affected by exogenous factors including environmental variables such as light intensity, light spectrum, mineral nutrition, plant architecture and temperature (Bernstein et al., 2019a, 2019b; Gorelick and Bernstein, 2014, 2017). Light is known to affect secondary metabolism by effects on both photosynthesis and photomorphogenesis (Bian et al., 2015; Landi et al., 2020). A need for standardization of the therapeutic compounds' profile, is therefore a major challenge in the development of cannabis for modern medicine, which requires mechanistic understanding of the regulation of secondary metabolite biosynthesis, as well as precision agriculture techniques for its standardization.

To facilitate cultivation under uniform environmental conditions and for generating a consistent product, there is a growing tendency for cultivation of cannabis in controlled growing rooms, termed 'in-door' cultivation in the cannabis jargon. In the 'in-door' facilities, light is supplied by artificial lighting. Since cannabis is a short-day plant, the supplied light needs to satisfy the photosynthetic energy demands as well as the spectrum required to trigger photoperiodic and photomorphogenetic responses. Several types of lights are being used nowadays in agricultural production systems, and in the cannabis industry, albeit very little science-based information is available on the response of cannabis to light spectrum for directing optimal precision agriculture practices. These include high intensity discharge lamps (HID) such as Metal Halide (MH) or High-pressure sodium (HPS); fluorescent lights and Light Emitting Diodes (LED). The lighting fixtures range in intensity and spectrum. Fluorescent fixtures vary in spectrum- with a high peak in the shorter wavelengths and decreased intensity with the increase in wavelength; HPS is red and far-red rich; MH is relatively uniform in intensity at all wavebands; and LEDs can be costume-made to cover desired regions of the spectrum (Wang et al., 2017). This study takes advantage of recent advances in LED technologies for generation of wavelength variations, for deciphering the effects of wavelength on cannabis growth, development, yield and chemical properties.

Very little and incomplete information is available about responses

of cannabis to light in general, and light quality in particular. Photosynthetic photon flux density up to 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ was found to increase net photosynthesis rate, and water use efficiency was highest under 500–1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Chandra et al., 2008). We have identified short day photoperiodism as the inducing trigger for morpho-development of inflorescences (Spitzer-Rimon et al., 2019). In addition, some light spectra effects were recorded both under long and short-day length. These include a slight reduction in height and leaf area under red + blue light compared to white light during long photoperiod (Lalge et al., 2017); lower plant and flowers biomass, but increased cannabinoids levels under HPS compared to two LED lightning systems (Magagnini et al., 2018); different chemical content under LED and florescence lighting (Namdar et al., 2019); and an increase in inflorescence yield with light intensity up to 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ regardless of lamp type used (LED and HPS) and spectra emitted (white light, red, blue), effects on the chemical profile were not reported (Eaves et al., 2020). Response to spectral changes varies between crops (Hogewoning et al., 2012) and cultivars, including effects on secondary metabolism (Tinyane et al., 2013) and it is therefore important to assess responses in cannabis of a diverse germplasm.

In this study we focused on the effect of light spectrum on 'drug-type' medicinal cannabis development and on the profile of cannabinoids, the unique therapeutic secondary metabolites in *cannabis sativa*. The hypotheses guiding the workplan were i. Light spectrum induces changes in the cannabinoid profile by affecting morpho-physiological responses. ii. The ratio of Blue:Red light induces changes in the cannabidiome. iii. Exposure to a wide (white light) spectrum at a given Blue:Red light ratio, enhances growth and cannabinoid concentration compared to exposure to only blue and red lights. iv. Genetic variability in the response to light spectrum will impact the cannabinoid profile. To test these hypotheses, we studied how four different light spectrums including white LED, two ratios of blue + red LED (1:4 and 1:1), and a spectrum generated by HPS (that is rich in the green to red wavelength range), affect the cannabidiome and morpho-physiological responses, comparatively in three cannabis cultivars differing in chemotype. In addition to the contribution to cannabis physiology, the acquired information can help develop precision-agriculture practices for increased specification and standardization of the chemical profile for the therapeutic modern medical cannabis industry.

2. Materials and methods

2.1. Plant material and growing conditions

Three 'drug-type' medical cannabis (*Cannabis sativa* L) genotypes differing in chemotype were used as the model system of study. CS10 is a high CBDA low THCA variety, CS12 produces similar concentrations of CBDA and THCA, and CS14 is a high THCA and low CBDA variety. The plants were propagated from cuttings in coconut fiber plugs (Easy plug CT 104, Goirle, the Netherlands). The rooted plantlets were planted in a 1 L square plastic pots in a growing mixture (50/50 coconut fibers/ perlite), 1 plant per pot, and were grown under long photoperiod of 18/6 light/dark hours for 5 days for vegetative growth. The plants were then transferred to a short-day regime (12/12 light/dark hours) in 4 growing chambers, and each chamber was illuminated by a different light-quality treatment. Five replicates plants per variety were grown in each chamber. The growing chambers size was 1.1 m width and depth and 2.0 m height, they were lined with reflective sheets, and contained an intake and discharge vents at the bottom and top, respectively, and a fan to enhance air circulation. The chambers were located in an environmentally controlled room, temperature was maintained at 25 °C, humidity at 50 % and CO₂ levels at 750–800 ppm. CS12, which is less vigorous compared to the other 2 varieties studied, was allowed a longer vegetative growth period of 24 days, under long photoperiod prior to the transition to short days. Fertigation was delivered from a final solution,

by drip irrigation, 1 dripper per pot, via 1.2 L h^{-1} drippers (Netafim, Tel Aviv, Israel), and the frequency of irrigations was adjusted from twice a week at the beginning of cultivation to once daily at late flowering. Fertilization was conducted with 'Coco forte' a and b, 1.65 ml L^{-1} each (Bionova, the Netherlands) with reverse osmosis desalinated water (Ampac, USA). EC of the fertigation solution was $1.7 \pm 0.05 \text{ mS cm}^{-1}$, and the pH was adjusted to 5.7 ± 0.1 , with H_3PO_4 .

2.2. Experimental treatments

The plants were exposed to 4 different light spectra that included 3 LED spectra compared to a control HPS light (Gavita pro 1000, Philips, the Netherlands), which is a conventional HPS standard light for commercial indoor cultivation. The LED treatments included a common white LED lightning system ("Fluence", Osram, Austin TX USA), which consists of mostly "white" LED light which has $\sim 1:1$ red and blue (Blue:Red) wavelength radiation ratio. The second LED treatment consisted as well of $\sim 1:1$ Blue:Red light but with little to none "white" light background, and the third LED treatment favored red light to a ratio of 1:4 Blue:Red (Fig. 1 Table 1). The treatments are named: HPS, White-LED, 1:1 LED, and 1:4 LED. The 1:1, and 1:4 LED treatments were generated by adjustable LED lightning systems ('EVA3', Flora Fotonica, Israel). Light intensity increased gradually from the beginning of the vegetative phase to reach $950 \mu\text{mol m}^{-2}\text{s}^{-1}$ at the photosynthetic active radiation (PAR) spectra fraction 10 days after the transition to short photoperiod. The HPS and 1:4 LED treatment emit light also in the infrared zone at the level of up to 25 % of the intensity given at the PAR range.

2.3. Chemical responses: cannabinoid concentrations

To evaluate effects of the different light spectra on the cannabinoid profile, apical inflorescences were sampled for cannabinoid analyses from the top of the main stem [primary inflorescence] and from the top of a bottom branch [secondary inflorescence]. The tissue was sampled for analyses 58 days after the transition to the short photoperiod, when about 40 % of the trichomes were of amber color. The inflorescences were hand trimmed, (i.e., tips of leaves protruding from the inflorescence were removed) as is routinely practiced in the cannabis industry. Trimming was conducted prior to drying immediately after dissection from the plant and fresh weight was determined. The inflorescences were dried in the dark at 40 % relative humidity and 19°C for 14 days, in an environmentally-controlled room. The analysis was conducted for all replicated plants per treatment, following the experimental design. For the cannabinoid analysis, the dried inflorescences were ground with a manual plastic grinder. Fifty mg of the ground tissue was placed in a glass vial, 10 mL ethanol was added, and the tube was shaken in a

Table 1

Spectral properties of the light treatments. Distribution of the light intensity in each treatment to spectrum ranges, presented as a percentage of the total light intensity. 1:1 and 1:4 represent two ratios of Blue:Red lights, supplied by LED.

Light spectra fraction (nm)	Light treatments			
	HPS (%)	LED White with $\sim 1:1$ (blue:red) (%)	LED 1:1 (blue:red) (%)	LED 1:4 (blue:red) (%)
300–400	0.05	0.00	0.26	0.00
400–500	4.51	25.5	47.1	18.4
500–600	40.7	40.2	3.30	3.13
600–700	33.9	32.9	48.2	71.3
700–800	4.34	0.80	0.98	7.00
>800	15.8	–	–	–
sum	99.2	99.5	99.8	99.9

reciprocal shaker for 1 h at room temperature. The extract was filtered through a polyvinylidene difluoride (PVDF) membrane filter of $0.22 \mu\text{m}$ pore size (Bar-Naor ltd, Ramat Gan, Israel). Cannabinoid concentrations in the filtered extracts were analyzed using a high performance liquid chromatography (HPLC) system, (Jasco 2000 Plus series) which consist of a quaternary pump, autosampler, column compartment and photo-diode array (PDA) detector (Jasco, Tokyo, Japan). The detection was carried out in a spectrum mode, at the wavelength range 200–650 nm. Chromatographic separations were conducted with a Luna Omega $3 \mu\text{m}$ Polar C18 column (Phenomenex, Torrance, CA USA) employing acetonitrile:water 75:25 (v/v) with 0.1 % (v/v) formic acid, at the isocratic mode, with a flow rate of 1.0 mL/minute. Quantification of cannabinoid concentrations were based on analytical standards: CBGA, CBN, CBD, CBDA (Sigma-Aldrich, Germany); and THCV, Δ^9 -THCA (THCA-A), Δ^9 -THC (Restek, Pennsylvania, USA). Concentrations of CBDV, CBG, THCV, CBCV, CBN, CBNA, Δ^8 -THC, CBL and CBT were below the detection limits. Linear R^2 of all calibration curves were >0.994 .

2.4. Plant morphological development and biomass accumulation

Non-destructive measurements of plant height were conducted routinely throughout the experiment, every 7–14 days. At the termination of the experiment the plants were dissected for the analyses of plant organ biomass. The plants were separated to fan leaves, stems and inflorescences, and each were weighted for fresh weight determination (Precisa 40SM-200A balance, Zurich, Switzerland). Inflorescences were air dried at 19°C and 40 % humidity following a conventional commercial practice. The tips of the inflorescence leaves protruding from the inflorescences were trimmed as is conventionally practiced for medical

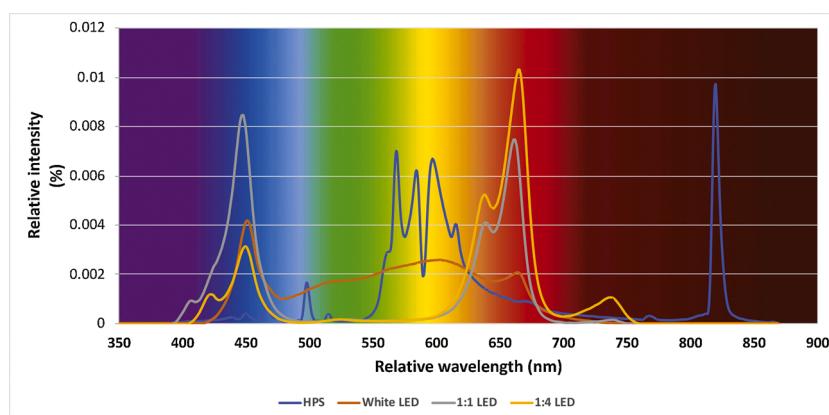


Fig. 1. Relative photon flux of spectral properties of the experimental treatments. 1:1 and 1:4 represent ratios of Blue:Red light, supplied by LED. HPS is high pressure sodium. Presented values are for 0.5 nm intervals.

and recreational use, and the biomass of the trim and the remaining inflorescences were determined.

2.5. Physiological responses

All physiological tests were conducted with 5 biological repeats (i.e., 5 replicated plants) per treatment, following the experimental design. The measurements were conducted on the youngest mature leaf on the main stem to facilitate comparison of tissue of similar physiological age. Reported results are averages \pm SE.

2.5.1. Photosynthetic pigments and gas exchange parameters

Concentrations of the photosynthetic pigments chlorophyll *a*, chlorophyll *b* and carotenoids were determined as described previously (Gorelick et al., 2015). In short, five discs 0.6 cm in diameter were cut from the youngest mature leaf on the main stem and kept in 80 % ethanol. Pigments were extracted following (Gorelick et al., 2015), and concentrations were calculated following Lichtenhaler and Wellburn (1983). Photosynthesis rate, transpiration rate, stomatal conductance, and intercellular CO₂ (Ci) were measured using a portable meter (LI-COR 6400XT LI-COR, Lincoln, NE, USA). Water use efficiency (WUEi) was calculated by dividing the assimilation rate by the transpiration rate as of Eq. (1) (Farquhar et al., 1982).

$$\text{Water use efficiency (\%)} = \frac{\text{photosynthesis rate}}{\text{transpiration rate}} * 100 \quad (1)$$

2.5.2. Membrane leakage

For membrane leakage measurements, the youngest mature leaf on the main stem was washed twice in distilled water, and the middle leaflet was shaken for 24 h in 50 mL distilled water in a closed vial. EC was measured twice, after 24 h of shaking, and again after autoclaving for 20 min in 120 °C and cooling to room-temperature. The percentage of ion leakage from the tissue, i.e., ‘Membrane leakage’, was calculated as the percentage of the first EC measurement results from the second measurement result.

2.5.3. Osmotic potential

About 200 mg of tissue from the center of the youngest mature leaf on the main stem was washed twice in distilled water, blotted dry, and placed into a 1.7 mL Eppendorf tube and immediately frozen. After partial thawing, the tissue was macerated in the vial and the cell sap was extracted by centrifugation at 6000 rpm and 4 °C for 5 min (Sigma Laboratory Centrifuges, Germany) as is described by Saloner et al. (2019). The osmotic potential of the sap was analyzed with a micro-osmometer (Gonotec, Berlin, Germany).

2.5.4. Relative water content

The second youngest mature leaf on the main stem was weighted for fresh weight and immersed in distilled water for 24 h. The leaf was then dried by blotting with paper towels, and weighted again for hydrated weight, and then dried at 65 °C for 3 days and was weighted for dry weight determination. Water content of the tissue was calculated by subtracting the dry weight of the tissue from the fresh weight. The hydrated water content is the dry weight subtracted from the hydrated weight. Relative water content of the tissue was calculated by dividing the fresh water content by the hydrated water content and multiplied by 100 as is described by Bernstein et al. (2019b).

2.6. Statistical analysis

The analysis was performed with the JMP software (version 9, SAS 2015, Cary, NC, USA). The data were subjected to one-way ANOVA ($\alpha < 0.05$) followed by Tukey's HSD test. The data met the assumptions of normality and homogeneity of variances.

3. Results

3.1. Chemical response

Cannabinoids are biosynthesized in the plants in acidic, carboxylated, forms that are later decarboxylated in the plant or post-harvest, to the active forms. Fig. 2 represents the combined concentrations of both carboxylated and decarboxylated forms of major cannabinoids and the percentage of decarboxylation. CBGA, the precursor of the major cannabinoids (THCA, CBDA and CBCA) was considerably affected by light spectra and demonstrated the highest variability and the most distinct response to light spectrum (Fig. 2A–C). The results demonstrate that blue light induces accumulation of CBGA in cannabis. Accordingly, White LED plants and 1:1 LED plants that had the highest proportions of blue light amongst all treatments, accumulated the highest levels of CBGA in all three varieties; plants exposed to the 1:4 LED treatment had similar or lower concentrations of CBGA, and plants cultivated under HPS light, which has the least amount blue light, always had the lowest concentration of CBGA (Fig. 2A–C). Unlike CBGA, concentrations of CBDA (Fig. 2D–F) were higher when grown with a low blue light ratio (HPS) than under high proportion of blue light in two of the varieties (CS10 and CS14). However interestingly, a contradictory response was apparent for CS12 plants that demonstrated lower CBDA accumulation when grown with low blue light supply (HPS, Fig. 2D–F) demonstrating a genotypic variability. Similarly, THCA (Fig. 2G–I) accumulation varied between the varieties with HPS decreasing THCA levels in CS14 and CS12, but increasing THCA levels in CS10. CBCA on the other hand (Fig. 2J–L), was least accumulated under the 1:4 LED treatment, but the differences between treatments were minor.

The extent of decarboxylation varied between the varieties and between cannabinoids but was not significantly affected by the light treatments. For example, the percentages of CBD out of the combined CBD + CBDA content in CS10 (high CBDA Fig. 2F) were 1.6–2.0 % in all treatments and at both locations. Similarly, THC in the balanced variety (CS12 Fig. 2H) ranged from 5.1–8.9 %. CS14 (high THCA) showed some variation from this trend and the extent of decarboxylation was greater for both THCA (4.1–10.7 %) and CBCA (24.5–40.9 %) (Fig. 2G, J). CBD levels for this variety were very low and under the detection limits. In most cases (68 % of the samples), the extent of decarboxylation was higher at the top [primary] inflorescence than the bottom [secondary] inflorescences. In addition, in most cases cannabinoid levels of the top inflorescence were higher than or similar to concentrations of the lower inflorescence. Four exceptions to this trend are the extent of decarboxylation of CBGA and CBCA in the 1:4 LED treatment in CS10 (the high CBD variety), and THCA levels of CS12 (balanced) and CS14 (high THCA) in the HPS treatment.

3.2. Morphological response

Photomorphogenetic response under short photoperiod was recorded by weekly height measurements (Fig. 3). For both the high THCA and high CBDA varieties (Fig. 3A, C) the tallest plants developed under the 1:4 LED spectrum, followed by the HPS. Both the 1:1 and the white LED treatments sustained shorter plants. Both lines increased in height during the first 19 days under short photoperiod by 40–50 cm, resulting in plants four to five times taller than at the start of the reproductive phase. During the following 34 days, the plants grew in height up to an additional 7 cm (with CS14 of the 1:4 treatment being an exception by growing an additional 17 cm). The morphological response of the balanced variety (Fig. 3B) differed, with red + blue LED treatments stimulating development of taller plants compared to both the HPS and white LED, and the extent of elongation over the flowering duration smaller compared to the other two lines (up to 17 cm). Cannabis is known to have a growth spurt at the beginning of the reproductive phase, with elongation of 10–200 % of their initial height, and this growth is cultivar specific. The duration of the vegetative period was

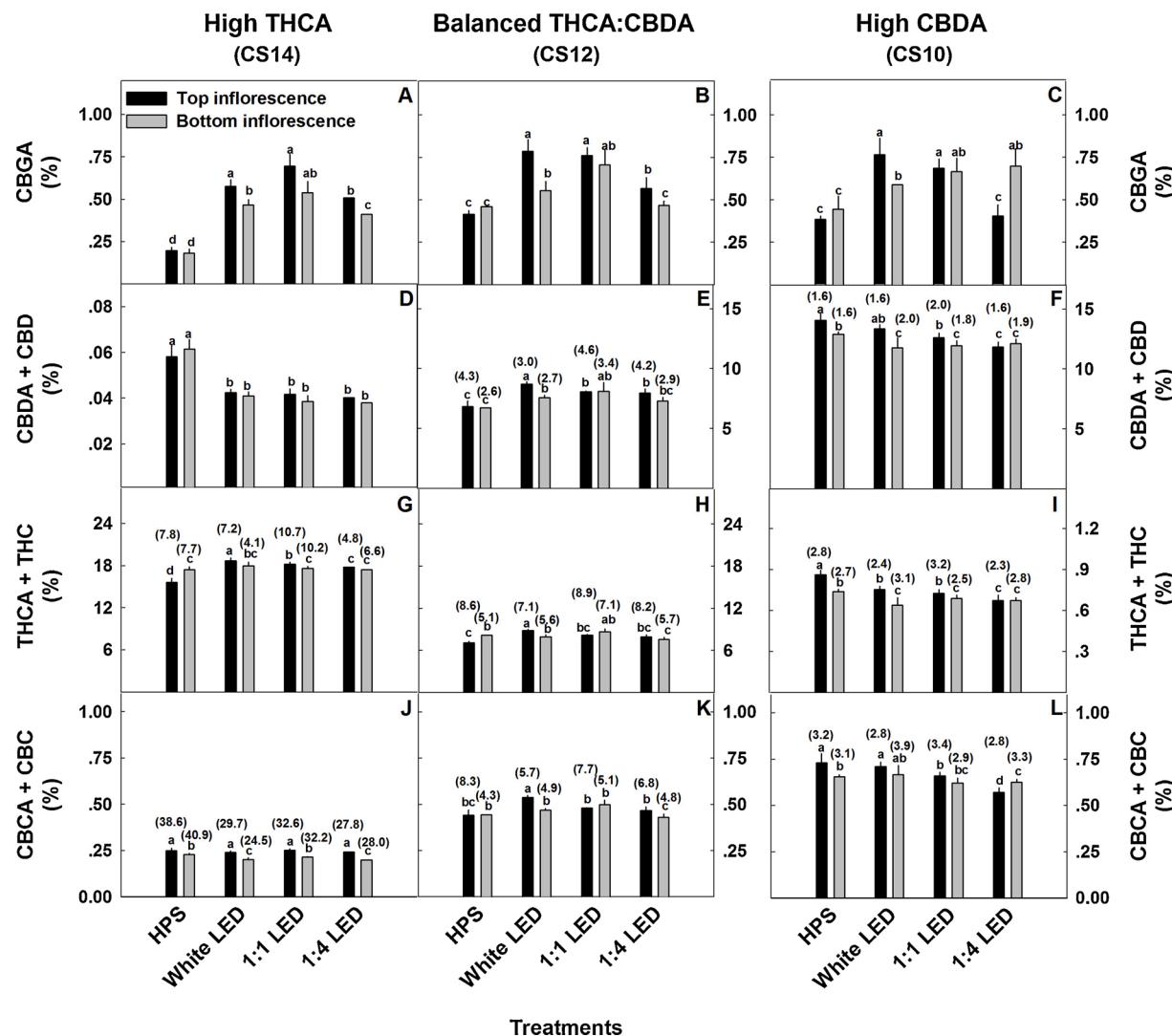


Fig. 2. Effect of light spectra on concentrations of major cannabinoids in primary inflorescences [top inflorescences] and secondary inflorescences [bottom inflorescences] in three medical cannabis varieties. A high THCA variety (CS14: A,D,G,F), a balanced CBDA:THCA variety (CS12: B,E,H,K), and a high CBDA variety (CS10: C,F,I,L). The numbers in parentheses above the bars represent the percentage of the decarboxylated form (out of the decarboxylated + carboxylated concentrations). Where the percentage is not presented, the decarboxylated form was not identified. The results are mean and SE ($n = 5$). Different letters above the bars represent significant differences between treatments by Tukey HSD test at $\alpha = 0.05$. Concentrations of CBDVA and THCVA ranged 0.02–0.6% and the response trends to the light treatments were very similar to CBDA and THCA, respectively (Fig. 1 supplemental).

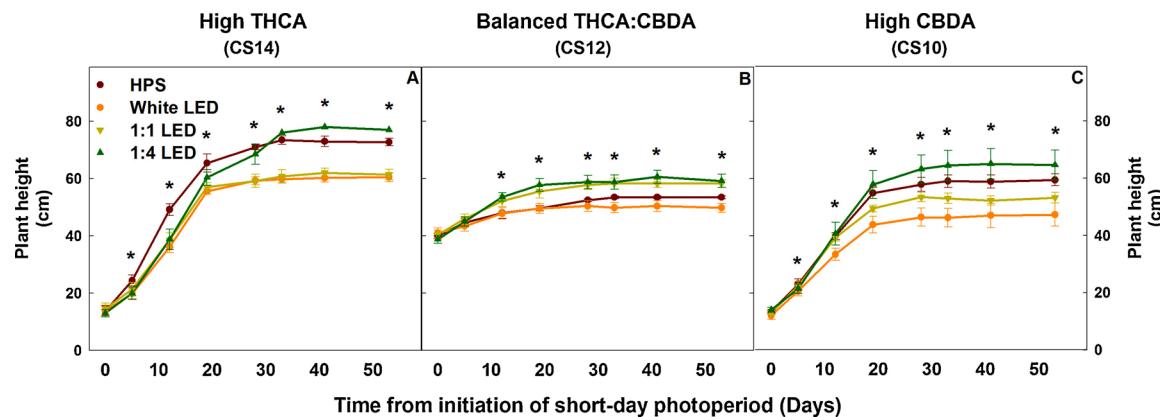


Fig. 3. Effect of light spectra on plant height throughout the short-day photoperiod, in three medical cannabis varieties: a high THCA variety (CS14 -A), a balanced THCA: CBDA variety (CS12 - B) and a high CBDA variety (CS10 -C). 1:1 and 1:4 represent ratios of Blue:Red light, supplied by LED. HPS is high pressure sodium. The results are mean \pm SE ($n = 5$). Asterisk above the averages represent a significant difference between treatments at the given day by Tukey HSD test at $\alpha = 0.05$.

adjusted for each cultivar based on prior knowledge of the elongation dynamic at the onset of the reproductive phase, to reach a similar final height of about 60 cm at maturity.

An additional morphological response that was affected by the light quality treatments is biomass deposition and partitioning between the plant organs. Plants of the 1:1 LED treatment always produced the most biomass, which was equal to some other treatments in the different cultivars. For example, similar flower biomass was developed by the white LED plants of the high THCA line (Fig. 4A); by the 1:4 LED plants in the balanced line (Fig. 4B); and by the 1:4 LED and HPS plants in the high CBDA line (Fig. 4C). Overall, biomass of the remaining plant organs responded similarly to the inflorescences biomass, with some statistically significant variations, such as different floral biomass in the white LED and 1:1 LED treatments in the high CBDA line.

3.3. Physiological characteristics

Photosynthesis rate (Fig. 5D-F) was highest in the 1:1 and 1:4 LED treatments in all three varieties and did not differ statistically from the rates obtained for HPS for CS10, and white LED for CS14. Stomatal conductance and transpiration rate responded similarly (Fig. 5A-C, G-I), with the 1:1 LED treatment allowing the greatest gas exchange, and for CS14 white LED had similar results. The lowest intracellular CO₂ levels (Fig. 5J-L) were always detected in the HPS plants, and in CS12 and CS10 also in the white LED plants. In 2 of the 3 studied lines (CS10 and CS12) the highest intercellular CO₂ concentrations were found in the 1:1 and 1:4 LED treatments, and in CS14 in the 1:1 and the white LED plants.

The plants also responded to the changing light spectrum by producing different concentrations of the light harvesting pigments chlorophyll a, chlorophyll b and carotenoids. Chlorophyll a (Fig. 6A-C) was most abundant in the 1:1 LED treatment in all varieties, while the least accumulation occurred in the HPS and 1:4 LED plants. The order of chlorophyll a abundance was 1:1 LED > white LED > HPS ≈ 1:4 LED. Similarly, the highest and lowest accumulation of carotenoids (Fig. 6G-I) occurred in the 1:1 LED and the HPS plants, respectively. Carotenoid concentrations under white LED did not differ significantly from the 1:1 plants' concentrations. The response of chlorophyll b production to the light treatment varied between the lines (Fig. 6D-F). It was unaffected by the treatments in CS12; was lowest in the 1:4 LED plants in both other lines, as well as in the HPS plants in the high THCA line.

The two water relations parameters tested, osmotic potential and RWC were unaffected by the treatments in the three lines tested (Fig. 7D-F, J-L). Similarly, membrane leakage, which is a stress measure of oxidative damage, was not affected by the treatments (Fig. 7A-C). 'Water use efficiency', which is derived from the stomatal conductance and photosynthetic rate results, was significantly affected by the light spectrum treatments. The most water efficient treatment was HPS, with white LED having similar results in both CS12 and CS10. The least water efficient treatment was always 1:1 LED, with white LED for CS14 and 1:4

LED for CS10 not-significantly differing from the 1:1 LED value.

4. Discussion

In the medicinal cannabis industry, yield and chemical profile has a major impact on patient welfare (Russo, 2019). Achieving certain attributes in the plant product, such as increased cannabinoids levels, or different ratio of CBDA to THCA can lead to a change in pharmaceutical potential and use (Gorelick and Bernstein, 2017). Secondary metabolism in plants, including cannabis, is influenced by environmental cultivation conditions (Bernstein et al., 2019a, 2019b; Saloner et al., 2019) including light. This study examined the hypothesis that changes in light spectra can induce a change in the chemical profile, as was observed for different crops (Landi et al., 2020; Ouzounis et al., 2015). The high number of plant blue and red light absorbing pigment super families (such as chlorophyll a,b (Qiu et al., 2019), Phytochrome a-e (Rockwell and Lagarias, 2020), cryptochrome 1-2 (Brudler et al., 2003), phototropin 1-2 (Briggs et al., 2001), along with results from studies on effects of these lights on plant development, suggest that the ratio between blue and red light, could have strong effects on plant development, especially, in term of yield quality and quantity.

In other species valued for their secondary metabolites, improved chemical traits were seen under exposure to different light spectra. In the medicinal plant Hypericum (*Hypericum perforatum*), elevated levels of hypericin, pseudohypericin and hyperforin were produced under red light compared to white or blue light (Nishimura et al., 2007). Sweet basil (*Ocimum basilicum*) leaves had increased levels of myrcene, 1,8-cineol and linalool when grown under monochromatic blue light compared to either white, red, green or blue-green light, but this increase was not observed for α-Pinene and β-pinene (Amaki et al., 2011) demonstrating quantitative as well qualitative effects. In the current study, we show that the cannabinoid profile was affected by the cultivation light spectrum. The largest impact was on the accumulation of the primary cannabinoid CBGA, that was up to 400 % higher in the LED treatments compared with the HPS control (Fig. 2A-C); while the concentrations of some of the primary cannabinoids (THCA, CDBA or CBCA) (Fig. 2D-L) decreased under LED cultivation but to a lesser extent (up to 40 % reduction), exhibiting metabolite specific response to light spectra, and a potential of inhibition of the metabolic enzymatic transformations under LED light. A similar response in scope was detected for cannabis by Magagnini et al. (2018), but higher cannabinoid concentrations, were measured under LED lighting compared with HPS. A less pronounced response to change in light quality in cannabis was shown by Hawley et al. (2018), though the differential light spectrum in this study was imposed by sub-canopy lighting (with a similar overhead spectrum, and some variability also in light intensity which may have masked the light quality effects). The reduced response was likely due to the smaller fraction of light that was altered, or due to a varied response to the monochromatic enhancement tested in the study.

Similar cannabinoid concentrations was seen in our study in the two

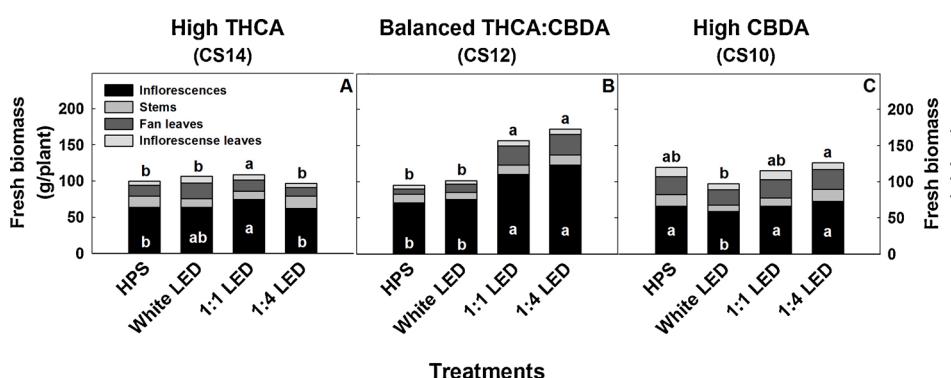


Fig. 4. Effect of the light spectral treatments on biomass of the plant organs in 3 medical cannabis varieties: CS14 (A), CS12 (B), CS10 (C). The results are mean ($n = 5$). 1:1 and 1:4 represent the ratios between blue:red light. Different letters above the bars represent significant differences between treatments for the combined biomass of the non-reproductive organs (i.e. fan leaves, inflorescence leaves and stems) by Tukey HSD test at $\alpha = 0.05$. Different letters within the bars represent significant differences between biomass of the reproductive organs (inflorescences) by Tukey HSD test at $\alpha = 0.05$.

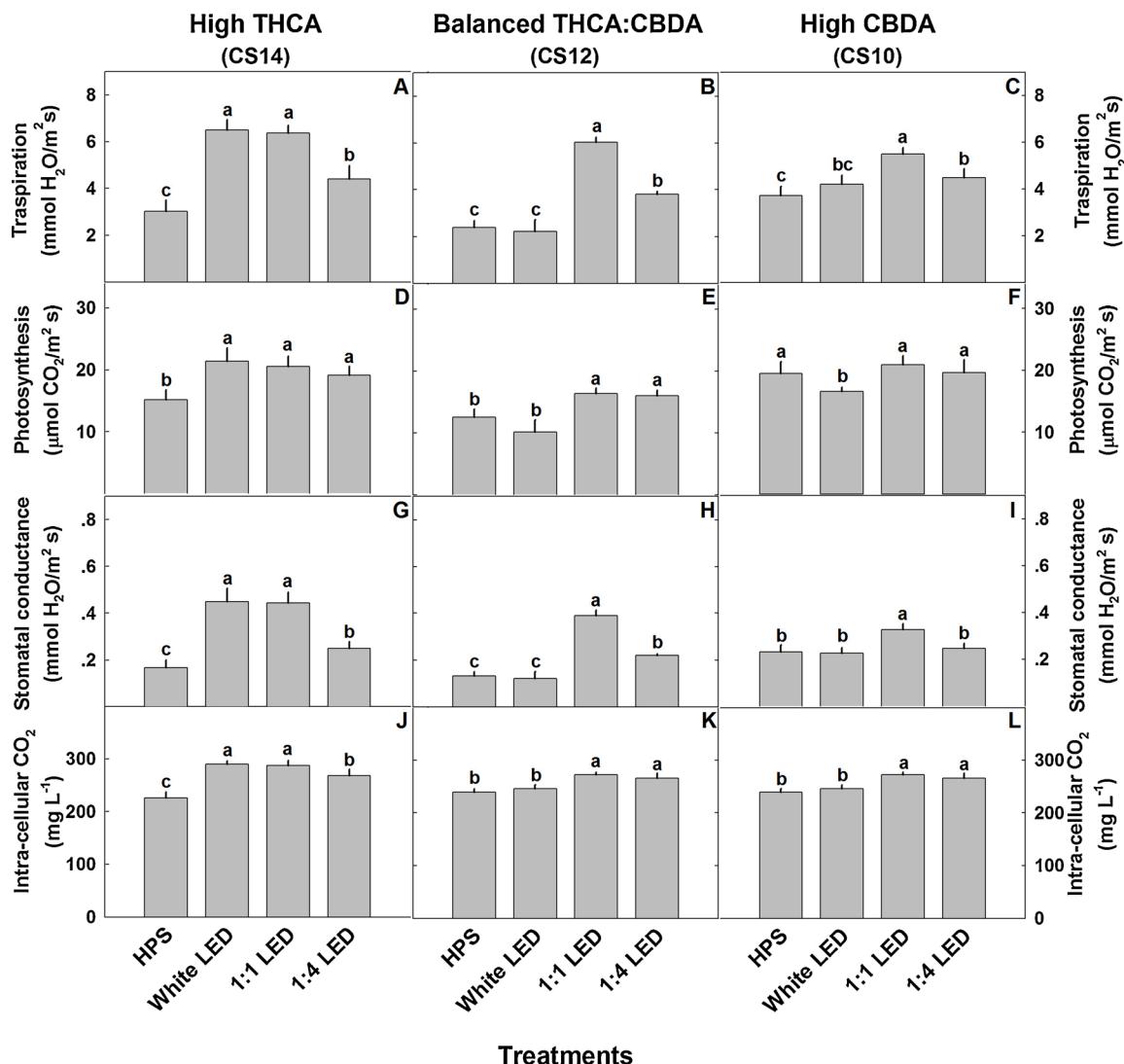


Fig. 5. Effect of light spectrum treatments on gas-exchange parameters in three medical cannabis varieties (CS14, CS12, CS10). Transpiration rate (A-C); photosynthesis rate (D-F); stomatal conductance (G-I); intra-cellular CO₂ (J-L). The results are means and SE ($n = 5$). Different letters above the bars represent significant differences between treatments by Tukey HSD test at $\alpha = 0.05$.

locals tested, i.e., primary and secondary inflorescences (Fig. 2). This likely reflects the small micro-climatic changes along the plants that were cultivated as is common for industrial indoor cultivation, i.e. small and non-dense plants which support homogenous light distribution. Commercial greenhouse cannabis cultivation usually includes bigger plants that shade one another resulting in micro-climate gradient within the canopy and specifically different spectral properties along the plant height. As leaves absorb mainly blue and red light, the light that reaches lower layers of the canopy is depleted in these zones of the spectrum, with mostly green and far-red light reaching the lower parts of the plant (Smith et al., 2017). These longitudinal variations in spectral properties along the plant axis, under both indoor and greenhouse cultivation, should be taken under consideration as light quality affects the cannabinoid profile. Hence, smaller, less dense plants and sub-canopy lighting have a potential to increase spectral and chemical uniformity.

Morphological development and physiological function of the cannabis plants were altered by light spectra (Figs. 3–7). Responses of plants to defined zones of the light spectrum are highly studied, and display variabilities as well as overlapping responses to different fractions of the spectrum. Blue light response, mediated through the cryptochrome and phototropins photoreceptors, include general responses such as de-etiolation, increased stomatal conductance and development,

photoperiodic flowering and circadian clock regulation, but also species specific responses such as leaf senescence in soybean (Yang et al., 2017). In Arabidopsis, lack of blue light promotes stem elongation as a shade avoidance response (Keller et al., 2011; Keuskamp et al., 2011). Similar responses in plants are also regulated by the phytochrome super family that react to the red: far-red (R:FR) ratio of the spectrum (Franklin and Quail, 2010).

High percentage of red light (that were applied in the 1:4 LED and HPS treatments) stimulated development of elongated plants (Fig. 3A–C), this is usually attributed to the activity of phytochromes which are the active receptors in the shade avoidance response (Franklin and Whitelam, 2005). HPS lights are rich in the far-red fraction of the spectrum and the HPS plants have elongated considerably, but the decreased R:FR ratio of the 1:4 treatment should have prevented stimulation of elongation growth, suggesting that at least partially, the lack of blue light is the cause of elongation in cannabis as described for other crops as well (Keller et al., 2011). In petunia (*Petunia X hybrida*), addition of blue light reduced elongation, while reduction of far-red and addition of red induced generation of the most compact plants (Gautam et al., 2015). These results partially correlate to the cannabis response, as lack of blue light stimulated elongation as seen for both the 1:4 LED and HPS treatments, as well as by the development of more compact

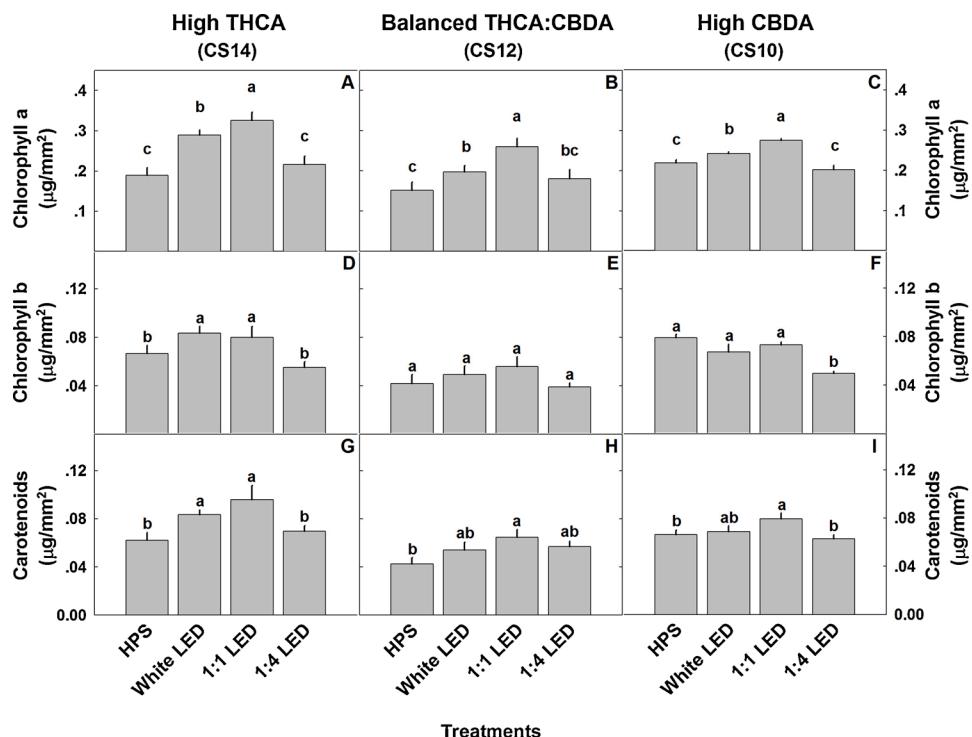


Fig. 6. Effect of light spectra treatments on concentrations of the photosynthetic pigments chlorophyll a (A-C); chlorophyll b (D-F) and carotenoids (G-I), in three medical cannabis varieties (SC10, SC12, SC14). The results are means and SE ($n = 5$). Different small letters above the bars represent significant differences between treatments by Tukey HSD test at $\alpha = 0.05$.

plants when grown under the 1:1 and white LED treatments. However, it contradicted it since the 1:4 LED treatment had a high R:FR ratio but resulted in elongated rather than compact plants. In roses and tomatoes, HPS light increased plant height compared to red and blue LED light (Blue:Red = 1:2), implying yet again that the high R:FR ratio combined with the low levels of blue cause plant stretching (Bergstrand et al., 2016). It is also possible that not only relative amount of blue light is the cause of such elongation response but rather the absolute amount. In soybean and radish for example, low amounts of blue light induced plant elongation, while under total higher irradiance plants remained at the same height even under different blue light ratios (Cope and Bugbee, 2013).

High biomass and yield are usually achieved by high photosynthesis rate which is most affected by light intensity, light absorption and photosynthetic efficiency (Qu et al., 2017). The 1:4 and 1:1 LED plants of 2 of the tested varieties showed higher photosynthesis rate (Fig. 5D-F), higher yield and total plant biomass compared with the white LED plants (Fig. 4). Similar to our results, in wheat, photosynthesis rate was highest under pink light- similar to 1:1 Blue:Red light, and lowest under cultivation with mostly blue light (75 % of the total PAR). Under fluorescent white light (that is closest to our white LED treatment) the assimilation rate was statistically similar to both a blue and a high red treatments (Blue:Red 1:5) that demonstrated low photosynthesis rates (Monostori et al., 2018). In the same study, biomass accumulation was similar in all treatments except the blue light, but yield was highest under exposure to red light with high far red ratio, and lower under pink, red (with low far red), and blue light, implying that light spectrum influences allocation of assimilates. In rice, different assimilation efficiencies were observed under different light intensities (Qu et al., 2017). According to Zhen and Bugbee (2020), far-red light should be taken under advise when considering PAR irradiance as it contributes to photosynthesis and biomass gain. Unfortunately, we did not detect such enhancing effect for cannabis, which is cultivated in many indoor facilities with far-red rich HPS lamps. Since photosynthesis is the source of organic matter for the plant, photosynthesis rate is expected to be reflected in biomass

accumulation. The lack of correlation between photosynthesis rate and biomass gain, which was apparent in our results for example for CS14 (that under 1:4 LED lights had high photosynthesis rate but low yield) (Figs. 4A and 5D), could be attributed to variability between treatments in respiration or biosynthesis of high-energy compounds. It cannot be excluded that this result may to some extent reflect the testing method, since at the instantaneous photosynthesis analyses the studied tissue was illuminated by the LI-COR measuring device with a unified light quality. Additionally, whole plant photosynthesis, light intensity and quality within the canopy may also play a part in whole plant development and especially in biomass and yield accumulation. Lastly, lower transpiration rate, which reduces intra-cellular CO₂ levels (Fig. 5A-C), as well as reduced photosynthetic pigment concentrations (Fig. 6) could have inhibited photosynthesis (Fig. 5D-F) at the whole plant level.

Increased yield could be also attributed to chlorophyll accumulation and CO₂ availability. Chlorophyll biosynthesis and stomatal conductance are both stimulated by phototropins activation by the blue wavelength light (Boccalandro et al., 2012; Im et al., 2006). In this study, we have used two treatments with high percentage of blue light (1:1 and White LED) and both stimulated pigment biosynthesis (Fig. 6) and transpiration (Fig. 5A-C). Similarly, higher chlorophyll content under LED (Blue:Red 1:2) compared to HPS light was seen in both roses and tomatoes (Bergstrand et al., 2016). The elevated pigment concentration likely facilitated the higher photosynthesis rate identified in this study, with the accompanied higher stomatal conductance facilitating sufficient aeration of the mesophyll tissue thus preventing limiting levels of CO₂ (Fig. 5K-L). In support of this finding, a linear relation between chlorophyll concentration and photosynthesis rate was reported before. For example, in papaya (*Carica papaya*), genotypes with lower chlorophyll concentrations had lower photosynthetic assimilation rates, biomass accumulation and yield (Paixão et al., 2019). It is possible that the differences in photosynthesis were larger than could have been detected by the measurements which utilized uniform instantaneous lighting at the pulse of the measurement period, suggesting a combined role for pigment concentrations and photosynthesis rate in the reduced

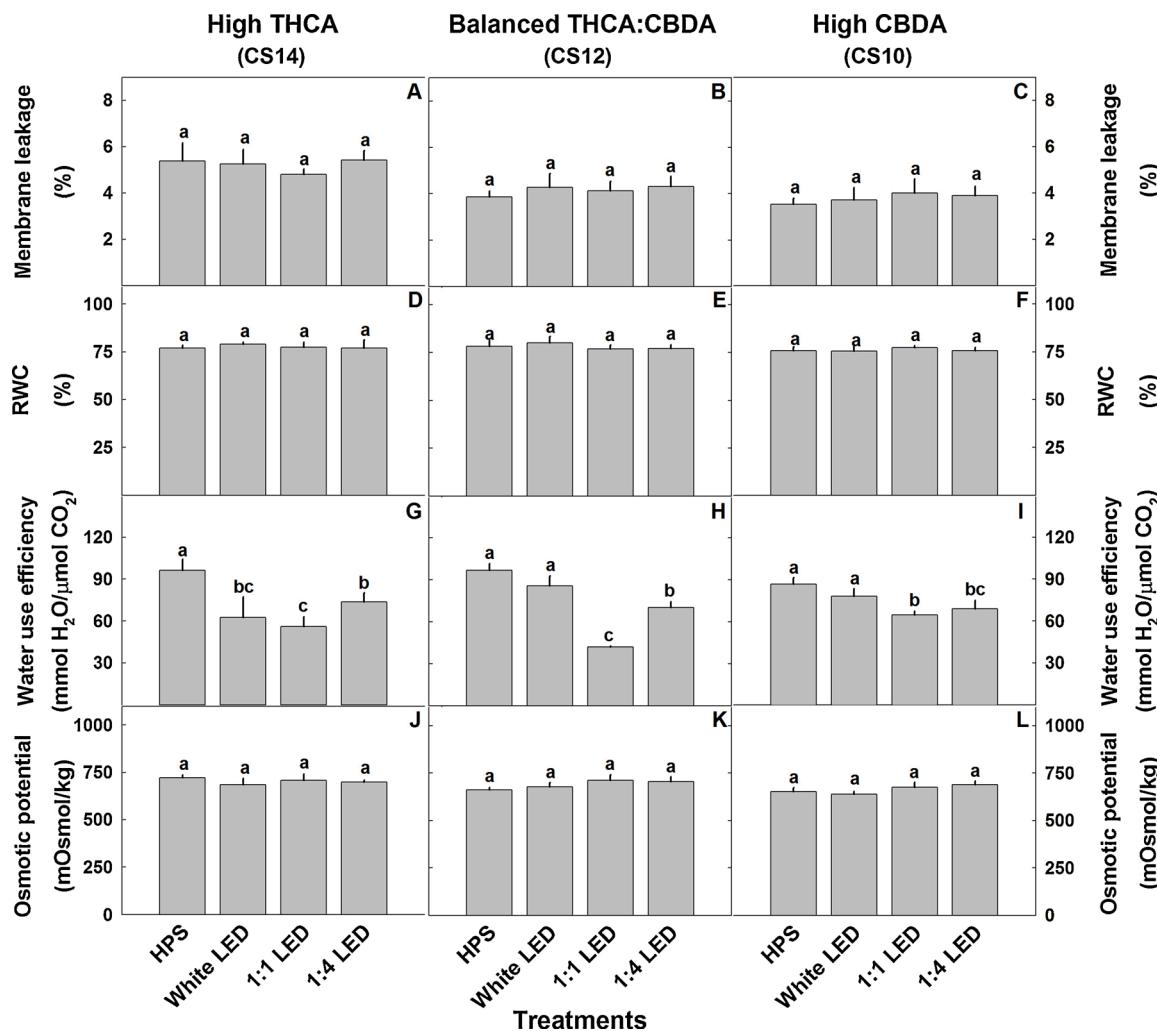


Fig. 7. Effect of light spectra on plant water-relations parameters in three medical cannabis varieties (CS10, CS12, CS14). Membrane leakage (A-C), Relative Water Content [RWC] (D-F), water use efficiency (G-I), and osmotic potential (J-L). The results are mean and SE ($n = 5$). Different letters above the bars represent significant differences between treatments by Tukey HSD test at $\alpha = 0.05$.

biomass deposition.

In a study by (Magagnini et al., 2018) a decrease in yield by LED light compared with HPS light was seen in one of two crop cycles. Such a decrease in yield was not seen in our study, nor in a study by Eaves et al. (2020) that observed a linear relation between light intensity and floral yield, which was not affected by light quality. As the results available so far suggest either increase, decrease or similar growth of inflorescences under different LED lights and spectra, further study of cultivar or spectra dependent effects is called for. Further analysis of light quality effects on photosynthesis along the plant height, during plant development and on a whole plant scale, are required to shed more light on cannabis physiological response and for directing optimization of cannabis cultivation.

5. Conclusions

Light spectrum affected almost all tested attributes in this study, including morphological, physiological and chemical parameters. Using three cultivars differing in chemotype, we found some cultivar-dependent responses, suggesting genetic variance, which opens up opportunities for breeding for enhanced light induced responses in cannabis. Furthermore, 2 water relations parameters, and an oxidative-stress related parameter, were not affected by the light quality treatments in all three varieties used, which implies that the light spectra has

low effect on cannabis water relations. Results of this study supported three of the proposed hypotheses including genetic variability in response to light spectrum, effect of Blue:Red light ratio on cannabis plant growth and the cannabinoid profile, but contradicted the hypothesis of a positive effect of full spectrum on yield. The results indicate that it is possible to alter the cannabinoid profile in cannabis by manipulation of the light spectrum, and suggest an interplay between genetics and light quality as a promising avenue for customizing the bioactive profile for the benefit of both patients and growers.

CRediT authorship contribution statement

Nirit Bernstein: Conceptualization, Methodology, Supervision, Writing - review & editing, Funding acquisition. **Nadav Danziger:** Data curation, Formal analysis, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.indcrop.2021.113351>.

References

- Amaki, W., Yamazaki, N., Ichimura, M., Watanabe, H., 2011. Effects of light quality on the growth and essential oil content in Sweet basil. *Acta Hortic* 907, 91–94. <https://doi.org/10.17660/ActaHortic.2011.907.9>.
- Andre, C.M., Hausman, J.F., Guerriero, G., 2016. Cannabis sativa: the plant of the thousand and one molecules. *Front. Plant Sci.* 7, 1–17. <https://doi.org/10.3389/fpls.2016.00019>.
- Bergstrand, K.J., Mortensen, L.M., Suthaparan, A., Gislerød, H.R., 2016. Acclimation of greenhouse crops to differing light quality. *Sci. Hortic.* (Amsterdam) 204, 1–7. <https://doi.org/10.1016/j.scienta.2016.03.035>.
- Bernstein, N., Gorelick, J., Koch, S., 2019a. Interplay between chemistry and morphology in medical cannabis (*Cannabis sativa* L.). *Ind. Crops Prod.* 129, 185–194. <https://doi.org/10.1016/j.indcrop.2018.11.039>.
- Bernstein, N., Gorelick, J., Zerahia, R., Koch, S., 2019b. Impact of N, P, K, and humic acid supplementation on the chemical profile of medical cannabis (*Cannabis sativa* L.). *Front. Plant Sci.* 10. <https://doi.org/10.3389/fpls.2019.00736>.
- Bian, Z.H., Yang, Q.C., Liu, W.K., 2015. Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: a review. *J. Sci. Food Agric.* 95, 869–877. <https://doi.org/10.1002/jsfa.6789>.
- Boccalandro, H.E., Giordano, C.V., Ploschuk, E.L., Piccoli, P.N., Bottini, R., Casal, J.J., 2012. Phototropins but not cryptochromes mediate the blue light-specific promotion of stomatal conductance, while both enhance photosynthesis and transpiration under full sunlight. *Plant Physiol.* 158, 1475–1484. <https://doi.org/10.1104/pp.111.187237>.
- Briggs, W.R., Beck, C.F., Cashmore, A.R., Christie, J.M., Hughes, J., Jarillo, J.A., Kagawa, T., Kanegae, H., Liscum, E., Nagatani, A., Okada, K., Salomon, M., Rüdiger, W., Sakai, T., Takano, M., Wada, M., Watson, J.C., 2001. The phototropin family of photoreceptors. *Plant Cell* 13, 993–997. <https://doi.org/10.1105/tpc.13.5.993>.
- Brudler, R., Hitomi, K., Daiyasu, H., Toh, H., Kucho, K.I., Ishiura, M., Kanehisa, M., Roberts, V.A., Todo, T., Tainer, J.A., Getzoff, E.D., 2003. Identification of a new cryptochrome class: structure, function, and evolution. *Mol. Cell* 11, 59–67. [https://doi.org/10.1016/S1097-2765\(03\)00008-X](https://doi.org/10.1016/S1097-2765(03)00008-X).
- Chandra, S., Lata, H., Khan, I.A., Elsohly, M.A., 2008. Photosynthetic response of *Cannabis sativa* L. to variations in photosynthetic photon flux densities, temperature and CO₂ conditions. *Physiol. Mol. Biol. Plants* 14, 299–306. <https://doi.org/10.1007/s12298-008-0027-x>.
- Cope, K.R., Bugbee, B., 2013. Spectral effects of three types of white light-emitting diodes on plant growth and development: absolute versus relative amounts of blue light. *HortScience* 48, 504–509. <https://doi.org/10.21273/hortsci.48.4.504>.
- Eaves, J., Eaves, S., Murphy, C., Murray, C., 2020. The relationship between light intensity, cannabis yields, and profitability. *Agron. J.* 112, 1466–1470. <https://doi.org/10.1002/agj2.20008>.
- Evans, J.R., Poorter, H., 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell Environ.* 24, 755–767. <https://doi.org/10.1046/j.1365-3040.2001.00724.x>.
- Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* 9, 121–137. <https://doi.org/10.1071/PP9820121>.
- Feng, L., Raza, M.A., Li, Z., Chen, Y., Khalid, M.H.Bin, Du, J., Liu, W., Wu, X., Song, C., Yu, L., Zhang, Z., Yuan, S., Yang, W., Yang, F., 2019. The influence of light intensity and leaf movement on photosynthesis characteristics and carbon balance of soybean. *Front. Plant Sci.* 9, 1–16. <https://doi.org/10.3389/fpls.2018.01952>.
- Franklin, K.A., Quail, P.H., 2010. Phytochrome functions in *Arabidopsis* development. *J. Exp. Bot.* 61, 11–24. <https://doi.org/10.1093/jxb/erp304>.
- Franklin, K.A., Whitelam, G.C., 2005. Phytochromes and shade-avoidance responses in plants. *Annu. Bot.* 96, 169–175. <https://doi.org/10.1093/aob/mci165>.
- Gautam, P., Terfa, M.T., Olsen, J.E., Torre, S., 2015. Red and blue light effects on morphology and flowering of *Petunia* × *hybrida*. *Sci. Hortic.* (Amsterdam) 184, 171–178. <https://doi.org/10.1016/j.scienta.2015.01.004>.
- Gorelick, J., Bernstein, N., 2014. Elicitation: an underutilized tool in the development of medicinal plants as a source of therapeutic secondary metabolites. *Adv. Agron.* 124, 201–230. <https://doi.org/10.1016/B978-0-12-800138-7.00005-X>. Elsevier Inc.
- Gorelick, J., Bernstein, N., 2017. Chemical and physical elicitation for enhanced cannabinoid production in cannabis. In: Chandra, S., Lata, H., ElSohly, M. (Eds.), *Cannabis sativa* L. - Botany and Biotechnology. Springer, Cham. Switzerland, pp. 439–456.
- Gorelick, J., Rosenberg, R., Smotrich, A., Hanus, L., Bernstein, N., 2015. Hypoglycemic activity of withanolides and elicited *Withania somnifera*. *Phytochemistry* 116, 283–289. <https://doi.org/10.1016/j.phytochem.2015.02.029>.
- Hawley, D., Graham, T., Stasiak, M., Dixon, M., 2018. Improving Cannabis bud quality and yield with subcanopy lighting. *HortScience* 53, 1593–1599. <https://doi.org/10.21273/HORTSCI13173-18>.
- Hogewoning, S.W., Trouwborst, G., Meinen, E., van Ieperen, W., 2012. Finding the optimal growth-light spectrum for greenhouse crops. *Acta Hortic.* 357–363.
- Huché-Thélier, L., Crespel, L., Le Gourrierec, J., Morel, P., Sakr, S., Leduc, N., 2016. Light signaling and plant responses to blue and UV radiations-perspectives for applications in horticulture. *Environ. Exp. Bot.* 121, 22–38. <https://doi.org/10.1016/j.enexpbot.2015.06.009>.
- Im, C.S., Eberhard, S., Huang, K., Beck, C.F., Grossman, A.R., 2006. Phototropin involvement in the expression of genes encoding chlorophyll and carotenoid biosynthesis enzymes and LHC apoproteins in *Chlamydomonas reinhardtii*. *Plant J.* 48, 1–16. <https://doi.org/10.1111/j.1365-313X.2006.02852.x>.
- Jackson, S.D., 2009. Plant responses to photoperiod. *New Phytol.* 181, 517–531. <https://doi.org/10.1111/j.1469-8137.2008.02681.x>.
- Kami, C., Lorrain, S., Hornitschek, P., Fankhauser, C., 2010. Light-regulated plant growth and development. *Curr. Top. Dev. Biol.* 91, 29–66. [https://doi.org/10.1016/S0070-2153\(10\)91002-8](https://doi.org/10.1016/S0070-2153(10)91002-8).
- Keller, M.M., Jaillais, Y., Pedmale, U.V., Moreno, J.E., Chory, J., Ballaré, C.L., 2011. Cryptochrome 1 and phytochrome B control shade-avoidance responses in *Arabidopsis* via partially independent hormonal cascades. *Plant J.* 67, 195–207. <https://doi.org/10.1111/j.1365-313X.2011.04598.x>.
- Keuskamp, D.H., Sasidharan, R., Vos, I., Peeters, A.J.M., Voesenek, L.A.C.J., Pierik, R., 2011. Blue-light-mediated shade avoidance requires combined auxin and brassinosteroid action in *Arabidopsis* seedlings. *Plant J.* 67, 208–217. <https://doi.org/10.1111/j.1365-313X.2011.04597.x>.
- Kim, H.H., Goins, G.D., Wheeler, R.M., Sager, J.C., 2004. Stomatal conductance of lettuce grown under or exposed to different light qualities. *Ann. Bot.* 94, 691–697. <https://doi.org/10.1093/aob/mch192>.
- Kong, Y., Zheng, Y., 2020. Phototropin is partly involved in blue-light-mediated stem elongation, flower initiation, and leaf expansion: a comparison of phenotypic responses between wild *Arabidopsis* and its phototropin mutants. *Environ. Exp. Bot.* 171, 103967. <https://doi.org/10.1016/j.enexpbot.2019.103967>.
- Lalge, A., Cerny, P., Trojan, V., Vyhnánek, T., 2017. The effects of red, blue and white light on the growth and development of *Cannabis sativa* L. *Mendel Net* 2017, 646–651.
- Landi, M., Zivcak, M., Sytar, O., Breštic, M., Allakhverdiev, S.I., 2020. Plasticity of photosynthetic processes and the accumulation of secondary metabolites in plants in response to monochromatic light environments: a review. *Biochim. Biophys. Acta Bioenerg.* 1861, 148131. <https://doi.org/10.1016/j.bbabiobio.2019.148131>.
- Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* 11, 591–592. <https://doi.org/10.1042/bst0110591>.
- Magagnini, G., Grassi, G., Kotiranta, S., 2018. The effect of light spectrum on the morphology and cannabinoid content of *Cannabis sativa* L. *Med. Cannabis Cannabinoids* 1, 19–27. <https://doi.org/10.1159/000489030>.
- Monostori, I., Heilmann, M., Kocsy, G., Rakcsig, M., Ahres, M., Altenbach, S.B., Szalai, G., Pál, M., Toldi, D., Simon-Sarkadi, L., Harnos, N., Galiba, G., Darko, É., 2018. LED lighting – modification of growth, metabolism, yield and flour composition in wheat by spectral quality and intensity. *Front. Plant Sci.* 9, 1–16. <https://doi.org/10.3389/fpls.2018.00605>.
- Nagy, J., Schäfer, E., 2002. Phytochromes control photomorphogenesis by differentially regulated, interacting signaling pathways in higher plants. *Annu. Rev. Plant Biol.* 53, 329–355. <https://doi.org/10.1146/annurev.arplant.53.100301.135302>.
- Namdar, D., Charuvi, D., Ajampura, V., Mazuz, M., Ion, A., Kamara, I., Kolтай, H., 2019. LED lighting affects the composition and biological activity of *Cannabis sativa* secondary metabolites. *Ind. Crops Prod.* 132, 177–185. <https://doi.org/10.1016/j.indcrop.2019.02.016>.
- Nishimura, T., Zobayed, S.M.A., Kozai, T., Goto, E., 2007. Medicinally important secondary metabolites and growth of *Hypericum perforatum* L. Plants as affected by light quality and intensity. *Environ. Control Biol.* <https://doi.org/10.2525/ecb.45.113>.
- Ouzounis, T., Rosenqvist, E., Ottosen, C.O., 2015. Spectral effects of artificial light on plant physiology and secondary metabolism: a review. *HortScience* 50, 1128–1135. <https://doi.org/10.21273/hortscl.50.8.1128>.
- Paixão, J.S., Da Silva, J.R., Ruas, K.F., Rodrigues, W.P., Filho, J.A.M., De Paula Bernardo, W., Abreu, D.P., Ferreira, L.S., Gonzalez, J.C., Griffin, K.L., Ramalho, J.C., Campostrini, E., 2019. Photosynthetic capacity, leaf respiration and growth in two papaya (*Carica papaya*) genotypes with different leaf chlorophyll concentrations. *AoB Plants* 11, 1–15. <https://doi.org/10.1093/aobpla/plz013>.
- Qiu, N.W., Jiang, D.C., Wang, X.S., Wang, B.S., Zhou, F., 2019. Advances in the members and biosynthesis of chlorophyll family. *Photosynthetica* 57, 974–984. <https://doi.org/10.32615/ps.2019.116>.
- Qu, M., Zheng, G., Hamdani, S., Essemene, J., Song, Q., Wang, H., Chu, C., Sirault, X., Zhu, X.G., 2017. Leaf photosynthetic parameters related to biomass accumulation in

- a global rice diversity survey. *Plant Physiol.* 175, 248–258. <https://doi.org/10.1104/pp.17.00332>.
- Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M., Chory, J., 1993. Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout arabidopsis development. *Plant Cell* 5, 147–157. <https://doi.org/10.1105/tpc.5.2.147>.
- Rockwell, N.C., Lagarias, J.C., 2020. Phytochrome evolution in 3D: deletion, duplication, and diversification. *New Phytol.* 225, 2283–2300. <https://doi.org/10.1111/nph.16240>.
- Russo, E.B., 2019. The case for the entourage effect and conventional breeding of clinical cannabis: No “Strain,” no gain. *Front. Plant Sci.* 9, 1–8. <https://doi.org/10.3389/fpls.2018.01969>.
- Saloner, A., Sacks, M.M., Bernstein, N., 2019. Response of medical cannabis (*Cannabis sativa* L.) genotypes to K supply under long photoperiod. *Front. Plant Sci.* 10, 1–16. <https://doi.org/10.3389/fpls.2019.01369>.
- Smith, H.L., Mcausland, L., Murchie, E.H., 2017. Don't ignore the green light: exploring diverse roles in plant processes. *J. Exp. Bot.* 68, 2099–2110. <https://doi.org/10.1093/jxb/erx098>.
- Song, Y.H., Shim, J.S., Kinmonth-Schultz, H.A., Imaizumi, T., 2015. Photoperiodic flowering: time measurement mechanisms in leaves. *Annu. Rev. Plant Biol.* 66, 441–464. <https://doi.org/10.1146/annurev-plant-043014-115555>.
- Spitzer-Rimon, B., Duchin, S., Bernstein, N., Kamenetsky, R., 2019. Architecture and florogenesis in female *Cannabis sativa* plants. *Front. Plant Sci.* 10, 350. <https://doi.org/10.3389/fpls.2019.00350>.
- Tinyane, P.P., Sivakumar, D., Soundy, P., 2013. Influence of photo-selective netting on fruit quality parameters and bioactive compounds in selected tomato cultivars. *Sci. Hortic. (Amsterdam)* 161, 340–349. <https://doi.org/10.1016/j.scientia.2013.06.024>.
- Wang, Y., Alonso, J.M., Ruan, X., 2017. A review of LED drivers and related technologies. *IEEE Trans. Ind. Electron.* 64, 5754–5765. <https://doi.org/10.1109/TIE.2017.2677335>.
- Yang, Z., Liu, B., Su, J., Liao, J., Lin, C., Oka, Y., 2017. Cryptochromes orchestrate transcription regulation of diverse blue light responses in plants. *Photochem. Photobiol.* 93, 112–127. <https://doi.org/10.1111/php.12663>.
- Zhen, S., Bugbee, B., 2020. Substituting Far-Red for traditionally defined photosynthetic photons results in equal canopy quantum yield for CO₂ fixation and increased photon capture during long-term studies: implications for Re-defining PAR. *Front. Plant Sci.* 11, 1–14. <https://doi.org/10.3389/fpls.2020.581156>.