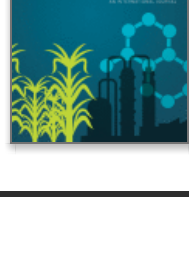


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Light matters: Effect of light spectra on cannabinoid profile and plant development of medical cannabis (*Cannabis sativa* L.)

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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.

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 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.

Section snippets

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 ...

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...

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)...

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...

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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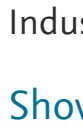
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