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Effects of Pulsed Light Spectra on Cannabinoid Accumulation in Thai *Cannabis sativa* L. 'Hang Kra Rog Phu Phan ST1'

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Abstract. Light is a very important component in indoor closed systems for medical cannabis production. In this research, we study a pulsed light spectral system that was established to stimulate the accumulation of the medically important compounds THC and CBD of Thai cannabis plants 'Hang Kra Rog Phu Phan ST1'. The cannabis callus used for the experiment was induced from cannabis saplings using 5 mg/L of 2,4-D MS agar medium, which was grown in a pulsed light incubator in a sterile condition. The developed pulsed light system consisted of a wavelength spectrum of 380 nm to 750 nm, and the amount of average photon flux density (PFD_{avg}) in the range of 350 –880 nm was between 112.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 604.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ which falls within the range of light intensities essential for stimulating THC and CBD production. The results have indicated that pulse frequencies of 50 Hz, 150 Hz, 400 Hz and continuous frequencies can stimulate accumulation of THC 1.66, 3.02, 3.80 and 11.11 ng/g callus and CBD 3.34, 3.37, 5.00 and 7.10 ng/g callus, respectively. In addition, pulsed light of 150 Hz at its duty cycle of 25%, 50% and 75% stimulated THC accumulation of 3.15, 3.02 and 5.55 ng/g callus and CBD 3.78, 3.37 and 4.06 ng/g callus, respectively. These results indicate that this pulsed light system can stimulate the accumulation of the medically important metabolites THC and CBD in cannabis calluses. The proposed research would offer the greatest benefits in medical cannabis production and pulsed light spectrum innovation.

Keywords: Callus, Cannabinoid, LED light, Pulsed light spectra, Tissue culture

1. Introduction

Cannabis (*Cannabis sativa* L.) has been used as a medicinal and herbal plant for thousands of years, as recorded in ancient Ayurvedic texts of various ethnic groups. Research has revealed that cannabis contains over 550 active compounds (ElSohly and Gul, 2014). Among them, the most important medicinal compounds are cannabinoids, primarily Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) (Andre et al., 2016; Anil et al., 2022). These substances exhibit a wide range of pharmacological properties, such as modulating the nervous system, hormonal balance, and immune response through the endocannabinoid system in the human body (Schrot and Hubbard, 2016; Rodriguez Mesa et al., 2021). Currently, cannabis is legally permitted for medical use and scientific research in several countries. As a result, the development of systems supporting the production of high-quality, medical-grade cannabis is becoming increasingly important. Medical cannabis should be cultivated in controlled indoor environments to ensure consistent quality. Variability in cannabis quality poses risks and uncertainties for both suppliers and users. Therefore, the production of medical-grade cannabis is typically

conducted under Good Manufacturing Practice (GMP) standards, which encompass all stages—from cultivation to processing and final product development.

Indoor plant production systems allow for precise environmental control, including light, temperature, humidity, and nutrient delivery. This enables consistent growth and accumulation of target compounds. Among these environmental factors, light plays a critical role in regulating plant development and secondary metabolite production in closed systems. In recent years, light systems for cannabis cultivation have been extensively studied. Research has focused on how light spectra influence phytochemical accumulation (Thirumurugan et al., 2018), including the effects of ultraviolet (UV) radiation—particularly UV-B (280–315 nm) and UV-A (320–400 nm)—and visible light (380–740 nm) on plants grown in greenhouses (Urban et al., 2016; Gupta et al., 2017; Alrifai et al., 2019). Lydon et al. (1987) showed that UV-B radiation enhances cannabinoid accumulation, while Magagnini et al. (2018) demonstrated that UV-A light can stimulate both the accumulation and increase of cannabinoids in cannabis. These findings clearly indicate that light spectra strongly influence the biosynthesis and accumulation of cannabinoids. However, excessively intense light may lead to saturation of light absorption in cannabis, which can reduce photosynthetic efficiency and growth.

The accumulation of medicinal compounds in cannabis depends not only on light spectra but also on genetic factors and the plant photoreceptors and enzymes (Eichhorn Bilodeau et al., 2019; Aliferis and Bernard-Perron, 2020; Vincent et al., 2021). Miliauskienė et al. (2021) found that pulsed LED light spectra—combining wavelengths of 450 nm, 520 nm, 660 nm, and 735 nm—with pulse frequencies of 0.5 kHz and 1 kHz were significantly more effective than continuous light in promoting the growth of baby leaf lettuce. Specifically, 0.5 kHz pulsed light enhanced plant performance by more than 30%, as measured by fresh weight, dry weight, and leaf area. This evidence suggests that both light spectra and pulsed light frequencies affect plant growth and the accumulation of key medicinal compounds. Therefore, this study aims to develop a pulsed light spectra system for stimulating the accumulation of THC and CBD in cannabis. The system is designed to be adjustable in both frequency and duty cycle (the ratio of pulse width to total period). The pulsed light includes wavelengths critical for growth and cannabinoid stimulation—specifically in the UV range (380–410 nm), red (660 nm), and far-red or infrared (760 nm), along with white light. The cannabis cultivar used in this study, Hang Kra Rog Phu Phan ST1, is a Thai local variety. The objective of this research is to identify the optimal pulse width-to-period ratio and pulsed light frequency that effectively enhance the accumulation of THC and CBD in this Thai cultivar. Additionally, the study aims to develop a prototype pulsed light system and generate foundational knowledge for improving the efficiency of medical cannabis production in the future.

2. Materials and Methods

2.1 Construction of a built-in pulsed light spectrum system within a growth chamber

The growth chamber was constructed using square metal tubing and a metal frame, divided into two compartments. Each chamber measured 40 × 75 cm. A LED light system was installed inside, consisting of 162 LED chips: 6 for UV light, 15 for red light, 6 for heat emission, and 131 for white light. A fan was also installed to maintain airflow. Electrical signal parameters—including pulse frequency, duty cycle, pulse intensity, and electrical current—were measured using an oscilloscope, a light sensor, and a multimeter. The internal space of the chamber was designed with a 5 cm buffer from the front and back edges. It was divided into 3 rows and 7 columns to position the callus samples. Each cell measured 10 × 10 cm, with a light intensity meter placed in one of the cells to measure the incident light. The LED system was configured to provide a photosynthetic photon flux density (PPFD) ranging from 50 to 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

2.2 Plant materials and tissue culture

The *Cannabis sativa* L. 'Hang Kra Rog Phu Phan ST1' was kindly provided by Rajamangala University of Technology Isan, Sakon Nakhon Campus and Medicinal Plant Research Institute, Department of Medical Sciences, Ministry of Public Health. In this study, we used *Cannabis sativa* L. callus culture techniques. All cannabis calluses were induced from seedlings and axillary buds in glass tissue culture bottles and grown on MS medium supplemented with 5 mg/L of 2,4-D under sterile conditions (Murashige and Skoog,1962; Logroño L. Javior, 2014). The samples were first incubated in darkness for 7 days, then transferred to a 16-hour light photoperiod for 60 days. The resulting cannabis tissues were subsequently subcultured onto fresh media for further experimentation.

2.3 Pulsed light spectrum system for the experiment

Experimental conditions were set from experimental chamber 1 to 6 by configuring the pulsed light system at frequencies of 50 Hz, 150 Hz, 400 Hz, 1000 Hz, 3000 Hz, and 5000 Hz. A duty cycle of 50% (ratio of pulse width to period) was applied, and the LED power supply was set to 100% light intensity. Young cannabis shoots cultured in tissue culture bottles were induced to form callus tissue. Data collection was carried out by visually observing and recording images of the changes that occurred. Once the callus reached a substantial size or was approximately 60 to 70 days old (when nutrient levels in the medium had significantly decreased), the best-performing samples were selected.

To investigate the stimulation of medically important compounds—THC and CBD—in cannabis callus derived from young shoots, various pulsed light frequencies were applied. The cannabis callus samples were transferred to fresh medium. The selected culture bottles were placed in the experimental chamber according to a predefined layout, while a separate set of control bottles was kept on standard tissue culture shelves under normal LED light. Experimental Chambers 1 through 6 were programmed with pulsed light at frequencies of 50 Hz, 150 Hz, 400 Hz, 1000 Hz, 3000 Hz, and 5000 Hz, respectively, using a 50% duty cycle (ratio of pulse width to period). Light intensity levels were adjusted using data obtained from observations to ensure optimal conditions. The growth of cannabis callus was monitored and recorded through image documentation. Once the callus reached a substantial size or was approximately 60 to 70 days old (when nutrient levels in the medium had significantly decreased), the best-performing samples were selected. A total of three bottles with well-developed callus were chosen and analyzed for the concentrations of the key medicinal compounds THC and CBD.

2.4 THC and CBD quantification

Four culture bottles containing well-developed cannabis callus were selected and used for the quantification of the key medicinal compounds, THC and CBD using UHPLC (High Performance Liquid Chromatography-DAD detector).

2.5 Data analysis

In this study, we investigated the following objectives: (1) to analyze the relationship between different pulsed light systems—varying in frequency and pulse width-to-period ratio—and the baseline pulsed light used in the experimental setup. (2) to analyze the relationship between the levels of key medicinal compounds (THC and CBD) and pulsed light intensity at different frequencies, using a fixed pulse width-to-period ratio of 50%, based on cannabis callus induced from young shoots. (3) to analyze the relationship between the levels of key medicinal compounds (THC and CBD) and pulsed light intensity at various frequencies and pulse width-to-period ratios, using well-developed cannabis callus samples.

3. Results

3.1 Optimization of a pulsed light spectrum system and a light-controlled experimental chamber

Each plant species has a unique system for capturing light and converting it into energy for growth and development. Previous studies have shown that light directly influences the accumulation of key chemical compounds in cannabis. Therefore, optimizing the light system for cannabis cultivation is crucial for maximizing production efficiency. In this study, we installed an LED light system inside a growth chamber (Figure 1A), consisting of 162 LED chips: 6 for UV light, 15 for red light, 6 for heat emission, and 131 for white light (Figure 1B). This LED system delivers an appropriate light spectrum ranging from 400 to 700 nm, which corresponds to the photosynthetically active radiation (PAR) range used by plants for photosynthesis.

The LED light system was optimized for this experiment by varying the pulse frequencies and the duty cycle (the ratio of pulse width to the total period). The average photosynthetic photon flux density (PPFD) and total photon flux density (PFD) for each setting were measured, as shown in Table 1. The results showed that at PWMs of 50, 150, 400, 1100, 3000, and 5000 Hz with a duty cycle (Dty) of 50%, the average photosynthetic photon flux density ($PPFD_{av\ g}$) was 186.9, 224.9, 365.9, 413.5, 424.5, and 421.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. The corresponding average total photon flux density ($PFD_{av\ g}$) values were 197.2, 237.5, 385.2, 436.2, 450.2, and 446.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Additionally, the measured pulsed frequencies were 49.50 Hz (Dty^{+} = 50%), 150.51 Hz (Dty^{+} = 50%), and 403.22 Hz (Dty^{+} = 76.67%) for PWMs of 50, 150, and 400 Hz, respectively. Thus, in this experiment, we developed a pulsed light spectral system capable of delivering sufficient light for cannabis growth and development, with frequencies ranging from as low as 50 Hz to continuous mode. The PFD_{avg} ranged from 112.4 to 604.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, depending on the PWM setting.

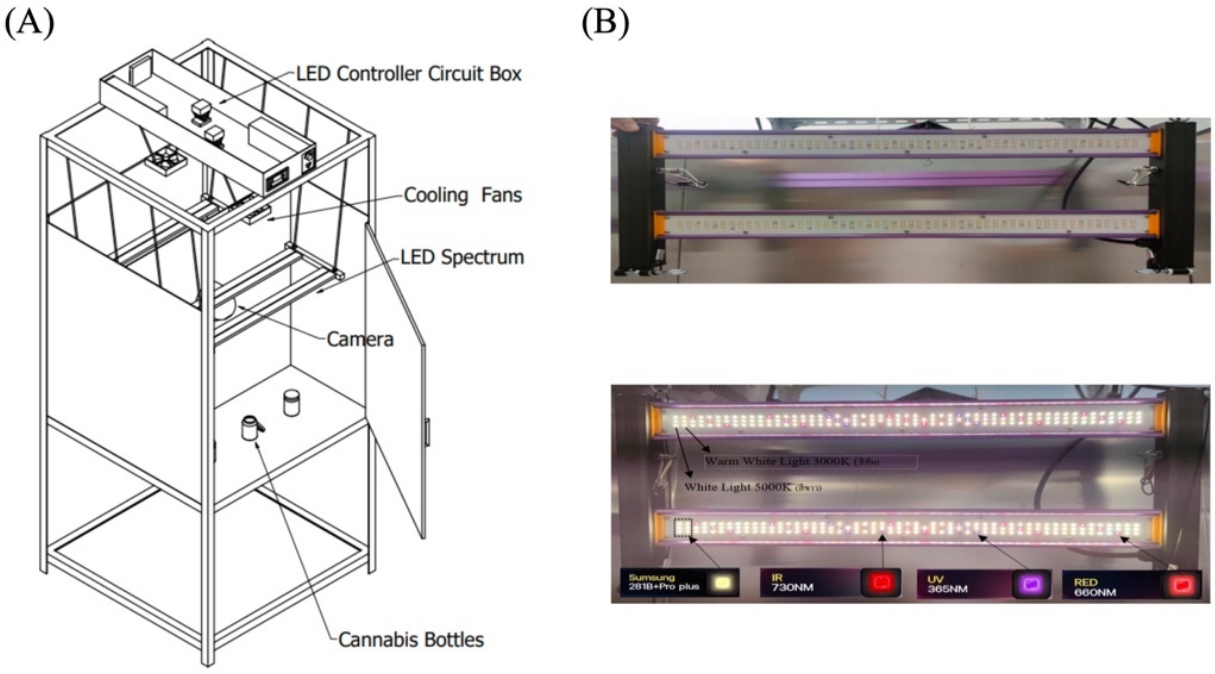

Figure 1. LED light system used in the cannabis growth chamber for this experiment. (A) 3D sketch of the experimental chamber. (B) The LED light system with lights on (top) and off (bottom).

Table 1. Optimization of the pulsed light spectral system used in this experiment to stimulate the accumulation of key chemical compounds (THC and CBD) in cannabis. The table lists the pulse width modulation (PWM), duty cycle (Dty), pulse frequency, average photosynthetic photon flux density (PPFD), and total photon flux density (PFD) of the LED system. Cont. indicates continuous light.

The LED light system		The pulsed LED light spectral system		$PPFD_{avg}$	PFD_{avg}
PWM(Hz)	Dty+:Dty- (%)	pulse fequency(Hz)	Dty+:Dty- (%)	($\mu mol \cdot m^{-2} \cdot s^{-1}$)	
50	50 : 50	49.50	50.14 : 49.85	186.9	197.2
50	50 : 50	49.50	50.14 : 49.85	326.5	350.6
150	25 : 75	151.51	24.50 : 75.49	106.1	112.4
150	50 : 50	151.51	50.37 : 49.62	224.9	237.5
150	50 : 50	151.51	50.37 : 49.62	448.5	469.0
150	75 : 25	151.51	74.36 : 25.63	586.6	604.8
400	50 : 50	403.22	76.67 : 23.32	365.1	385.2
400	50 : 50	403.22	49.92 : 50.08	487.3	517.5
1100	50 : 50	Cont.	Cont.	413.5	436.2
1100	50 : 50	Cont.	Cont.	525.5	555.8
3000	50 : 50	Cont.	Cont.	424.5	450.2
3000	50 : 50	Cont.	Cont.	520.0	550.0
5000	50 : 50	Cont.	Cont.	421.2	446.6
5000	50 : 50	Cont.	Cont.	536.7	569.8

3.2 The effect of a pulsed light spectral system on the development of cannabis callus: Hang Kra Rog Phu Phan ST1

To investigate the influence of our pulsed light system on cannabis callus growth, callus cultures on MS solid media in bottles were placed at different positions within six growth chambers (Figure 2A, 2B). The pulsed frequencies were set at 50, 150, 400 Hz, corresponding to PWM settings at 1000, 3000, and 5000 Hz, respectively with a duty cycle of 50%. The total photon flux density (PFD_{avg}) at each bottle position was measured. Callus cultures were selected for further study based on the following criteria: two samples were taken from both clone A and B, and all selected callus cultures showed better development compared to the others. At 74 days after exposure to the pulsed light system, the cannabis callus developed a darker green color. In addition, selected samples (sample no. 7 and 10) were roughly measured, revealing that the callus exhibited horizontal growth of approximately 1.0–1.1 times and vertical growth of 1.2–1.4 times compared to their initial dimensions (Figure 2C). The light intensity measured as PFD_{avg} at the positions of the selected samples ranged from 382.8 to 624.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table 2). These results indicate that the pulsed light system had a slight impact on the size of cannabis callus.

3.3 The effect of a pulsed light spectral system on the chemical compounds in cannabis callus: Hang Kra Rog Phu Phan ST1

To investigate the stimulatory effect of a pulsed light system on the chemical compounds THC and CBD, callus samples were selected and positioned under the system as shown in Figure 2A. Pulse frequencies were varied at 50, 150, 400, 1000, 3000, and 5000 Hz to determine the optimal frequency range affecting the accumulation of THC and CBD in cannabis callus. After 74 days, the samples were analyzed for THC and CBD content using ultra-high performance liquid chromatography with a diode array detector (UHPLC-DAD). The results indicated that a pulse frequency of 150 Hz with a duty cycle of 50% produced the highest levels of

THC (8.19 ng/g callus) and CBD (4.42 ng/g callus). (Table 3).

Next, we investigated the effect of a pulsed light system on well-developed (older) cannabis callus. Callus that had been grown under normal LED light for 5 months (150 days) was transferred and cultivated under the pulsed light system at frequencies of 50, 150, and 400 Hz, corresponding to a PWM value of 1100 Hz and a duty cycle of 50%. In addition, at 150 Hz, the duty cycle was varied at 25%, 50%, and 75%. After 30 days under these conditions, callus samples were selected for THC and CBD quantification using UHPLC-DAD (Table 4). The results showed that pulse frequencies of 50 Hz, 150 Hz, 400 Hz, and continuous light induced THC accumulation at levels of 1.66, 3.02, 3.80, and 11.11 ng/g callus, respectively, and CBD accumulation at 3.34, 3.37, 5.00, and 7.10 ng/g callus, respectively. Furthermore, at a pulse frequency of 150 Hz with varying duty cycles of 25%, 50%, and 75%, THC production was 3.15, 3.02, and 5.55 ng/g callus, while CBD production was 3.78, 3.37, and 4.06 ng/g callus, respectively. Our results also showed that the highest levels of THC and CBD were induced under continuous light conditions. However, under this condition, the total callus weight was the lowest—only 10.32 g.

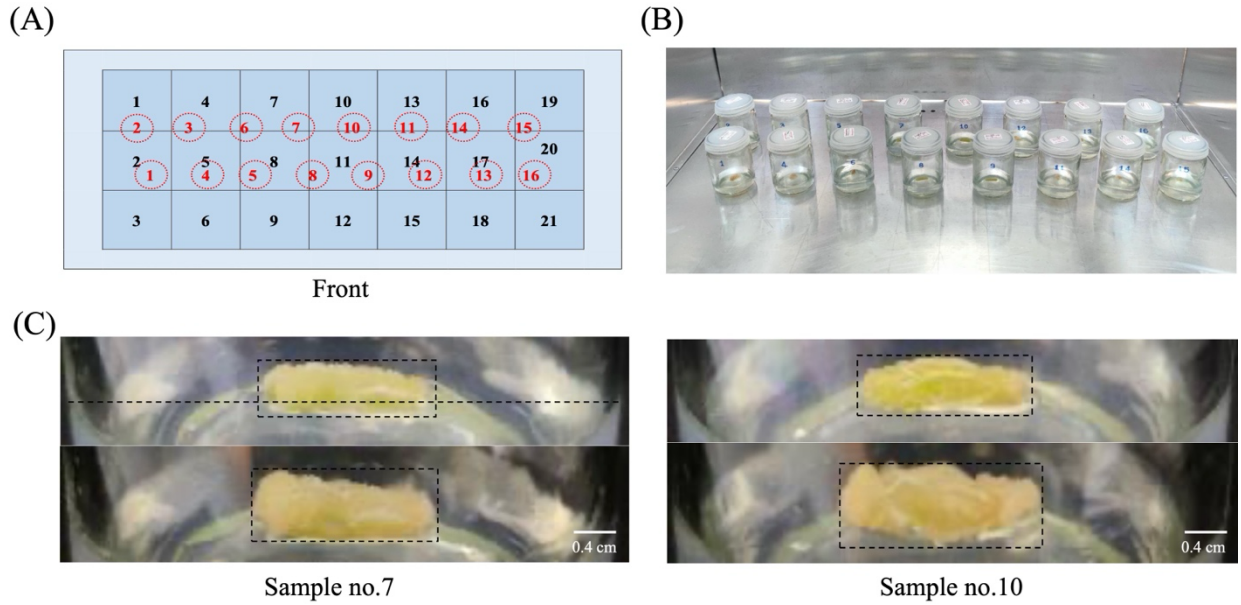

Figure 2. The effect of a pulsed light spectral system on the development of cannabis callus. (A) Map showing the positions of cannabis callus inside the growth chamber. (B) A representative photo of cannabis callus inside the chamber. (C) Selected callus samples showing size differences at the beginning of the experiment (top) and after 74 days of exposure to the pulsed light system (bottom).

Table 2. Selected positions of cannabis callus samples chosen for further investigation, along with the measured total average photon flux density (PFD_{avg}) at each position. The position numbers within the growth chamber correspond to the map shown in Figure 2A

Chamber No.	Position No.	PWM (Hz)	PFD_{avg} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
1	7, 8, 9, 10	5000	624.2
2	7, 8, 9, 10	400	605.6
3	5, 6, 7, 8	150	520.8
4	7, 8, 9, 10	50	382.8
5	7, 8, 9, 10	1100	607.2
6	7, 8, 9, 10	3000	603.3

Table 3. Quantification of THC and CBD at different pulse frequencies. Values are expressed in nanograms per gram (ng/g) of cannabis callus. Cont. indicates continuous light.

PWM	Pulse Spectra of LED system		Callus weight (g)	THC (ng/g callus)	CBD (ng/g callus)
Frequency (Hz)	Frequency (Hz)	PFD_{avg} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)			
50	50	219.6	3.55	5.82	-
150	150	263.8	2.55	8.19	4.42
400	400	423.0	5.64	3.56	0.79
1100	Cont.	478.9	3.47	-	-
3000	Cont.	487.8	3.83	-	2.14
5000	Cont.	490.3	2.60	-	-

Table 4. Quantification of THC and CBD of well-developed cannabis callus used as starter material at different pulse frequencies. Values are expressed in nanograms per gram (ng/g) of cannabis callus. Cont. indicates continuous light.

PWM		Pulse Spectra of LED system		Callus weight (g)	THC (ng/g callus)	CBD (ng/g callus)
Frequency (Hz)	Dyt+:Dyt-	Frequency (Hz)	PFD_{avg} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)			
1100 (Initiation stage)	50:50	Cont.	496.8	10.76	-	-
50	50:50	50	371.4	13.99	1.66	3.34
150	25:75	150	118.5	11.98	3.15	3.78
150	50:50	150	505.4	14.36	3.02	3.37
150	75:25	150	644.7	11.09	5.55	4.06
400	50:50	400	562.0	14.10	3.80	5.00
1100	50:50	Cont.	594.8	10.32	11.11	7.10

4. Discussion

This study demonstrates the feasibility and effectiveness of using a pulsed light spectral system to modulate the growth and chemical composition of cannabis callus, specifically targeting the accumulation of tetrahydrocannabinol (THC) and cannabidiol (CBD). Through the development and optimization of an LED-based growth chamber, we were able to deliver a controlled spectrum of light within the photosynthetically active radiation (PAR) range (400–700 nm), with adjustable pulse frequency and duty cycle. This allowed for fine-tuning of the light environment and provided a foundation for exploring the effects of dynamic light conditions on plant tissue cultures.

In terms of callus growth (Table 2), the results showed that pulsed light exposure, particularly at moderate PFD levels (382.8–624.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$), slightly promoted vertical and horizontal expansion of cannabis callus compared to the initial stage. Callus subjected to pulsed light developed a deeper green color, suggesting enhanced production of photosynthetic pigments. This observation aligns with recent studies showing that light quality and intensity significantly influence the vegetative growth of cannabis. For example, the cannabis 'Yunma 1' exhibited increased stem diameter and higher root dry and fresh weights under LED light intensities, with optimal values observed at 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Interestingly, plant height decreased as light intensity increased (Roman et al., 2024). Similarly, Westmoreland et al. (2021) reported that cannabis yield was highest at a light intensity of 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$; however, this level did not significantly affect cannabinoid content. In addition, for other varieties such as Gelato (an indica-dominant hybrid), key morphological parameters were optimized at 600–900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Moher et al., 2022). These findings indicate that the effects of light intensity are dependent on the specific cannabis genotype.

The chemical substances, THC and CBD, were further quantified to assess the effect of pulsed light system on improving the compound accumulation. In younger callus (74-day-old samples), the pulsed light system at 150 Hz and 50% duty cycle resulted in the highest combined levels of THC (8.19 ng/g) and CBD (4.42 ng/g), suggesting this frequency may fall within a stimulatory range for cannabinoid biosynthesis. Interestingly, continuous light conditions produced even higher concentrations of cannabinoids in well-developed callus samples (11.11 ng/g THC and 7.10 ng/g CBD), but also resulted in significantly lower biomass (10.32 g). These results suggest that light quality strongly affects cannabinoid accumulation, which is in line with previous studies (Amrein et al., 2020; Danziger & Bernstein, 2021). Moreover, the increased cannabinoid levels under low-biomass conditions may imply a stress-related enhancement in secondary metabolism. This observation is consistent with earlier findings indicating that high light exposure or environmental stress can trigger the production of bioactive compounds in plant tissues (Caplan et al., 2019; Islam et al., 2021). When evaluating different duty cycles at 150 Hz, we observed that higher light exposure (75% duty cycle) increased both THC and CBD levels, particularly in older callus, with cannabinoid accumulation rising alongside photon flux density (PFD). This suggests a light dose-dependent response, where increased photonic energy stimulates metabolic activity up to a certain threshold as previously highlight in several studies (Llewellyn et al., 2022; Moher et al., 2022; Ahsan et al., 2024).

Overall, these results highlight that light frequency, intensity, and pulse duration all interact to influence cannabinoid production. While continuous light maximized secondary metabolite accumulation, it negatively impacted biomass, which may not be ideal for commercial production. Pulsed light, particularly at 150 Hz with optimized duty cycles, may offer a compromise by moderately enhancing cannabinoid yield while preserving growth. Further research is needed to understand the molecular mechanisms behind these light-mediated responses, including the roles of photoreceptors, gene expression of biosynthetic enzymes, and potential oxidative stress responses. This knowledge could support the development of optimized light regimes tailored for cannabinoid production in vitro, offering a non-chemical approach to enhancing the value of tissue culture-derived cannabis products.

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