The Impact of Duty Cycle and Frequency of Pulse LED Lighting as a Sustainable Energy Saving Option to Reduce the Cost per Kilogram of Vertical Farming

Thanujika Wickramaachchi (23855098)

Supervisors: Assoc Prof. Nicolas L Taylor and Mr. Alex Montanari

Chosen Journal: Journal of Plant Biotechnology

This paper is submitted in partial fulfilment of the requirements for a

Master of Biotechnology (71580)

SCIE5571-SCIE5574

School of Molecular Sciences

Faculty of Science

The University of Western Australia

October 2024

Word Count: 5070

ACKNOWLEDGMENTS

I would like to express my deepest appreciation to my supervisors, Assoc Prof. Nic and Mr. Alex, who introduced me to pulse lighting, which has fascinated me deeply and provided me with a profound understanding of the subject. I also express my sincere gratitude to Agyeya, whose invaluable support and direction have been beneficial despite his busy schedule. Finally, I am profoundly grateful to my parents and friends for their unwavering support and constant encouragement throughout my academic journey. This accomplishment would not have been possible without them. Thank you.

ABSTRACT

The study investigated the effects of different pulsed light treatments on the growth parameters of basil plants and their photosynthetic light utilisation to reduce production costs in vertical farming systems. The experiment compared the impact of continuous and pulsed lighting on basil yield, maintaining a constant photosynthetic photon flux density (PPFD) of 250 μmol m^{-2} s^{-1}, with variations in average PPFD according to the duty cycle. Pulsed irradiation was applied by adjusting duty cycles and frequencies: 85% duty cycle at 0.05 kHz, 75% duty cycle at 1 kHz, 50% duty cycle at 0.5 kHz, and 50% duty cycle at 20 kHz. The results demonstrated that basil plants grown under pulsed lighting exhibited superior growth performance, particularly in terms of fresh and dry biomass. However, no significant differences in stomatal conductance and the quantum yield of photosystem II (PSII) were observed between the control and pulse-treated groups, except for the 75% duty cycle at 1 kHz treatment. Power consumption was reduced in the pulsed lighting treatments compared to continuous lighting, although the reduction did not strictly correlate with the duty cycle, as frequency also played a role. Additionally, light use efficiency was higher in basil plants subjected to pulsed lighting than those under continuous light. The findings suggest that the most cost-effective pulsed light treatment for basil production, in terms of cost per kilogram, was a 50% duty cycle at 20 kHz. In conclusion, pulsed LED lighting with varying duty cycles and frequencies did not significantly inhibit basil growth, indicating that this technology has the potential to reduce energy consumption in crop production within vertical farming systems.

Keywords: Pulse lighting, duty cycle, frequency, photosynthetic photon flux density, quantum yield

1. Introduction

1. Background

The world's population is predicted to increase from 7.7 billion in 2019 to 9.7 billion in 2050, an increase of more than 25%. A 70% rise in food production is needed to feed this expanding population (Wang et al., 2021). Recent research indicates that global food production has a significant environmental impact on Earth. It utilises almost 40% of the world's arable land, consumes 70% of extracted water and is responsible for 30-34% of greenhouse gas emissions (German et al., 2017). More sustainable plant production methods must be developed to ensure ongoing supply and safeguard food security.

Vertical farming, a type of controlled environment agriculture, has garnered attention as an alternative approach to feeding a growing population while reducing the environmental impact of food production (van Iersel et al., 2016). This method of plant production involves growing crops in a warehouse-like structure that is nearly airtight and thermally insulated. This structure typically contains many vertically stacked culture shelves with electric lamps (Kozai et al., 2019). Additional equipment, such as an environmental control unit, air circulation fans, air conditioners and supply units for nutritional solutions and CO_2, may be required to maintain optimal environmental conditions. LED lights have become increasingly popular in modern vertical farms due to their small size, low lamp surface temperature, excellent light use efficiency, and broad light spectrum. By stacking more culture shelves vertically, land use efficiency is improved (Kozai et al., 2019), which means that these farms have the potential to produce 200 to 1000 times more food per unit of land area than conventional agriculture. However, constructing and operating vertical farms can be expensive, and the biomass produced must be sold for ~ $13.75/kg to cover the production costs (van Iersel et al., 2016). Existing research focuses on maximising yield, but commercial production requires reduced price per kilogram of product to provide a viable alternative to traditional agriculture. Lighting and HVAC (heating, ventilation and air conditioning) constitute the most significant proportion of the production costs and thus present the greatest opportunity for cost reduction.

Photosynthesis in enclosed plant factories is solely powered by electric light, and LEDs are a standard light source used in vertical farming (van Iersel et al., 2016). Electricity is the primary ongoing expense for large vertical farms, with over 30% of the operation's total cost attributed to powering and cooling LED lights (van Iersel et al., 2016). Significant and sudden variations in irradiance are commonplace for plants or leaves in their natural state. Under this fluctuating irradiation, photosynthetic performance differs from steady-state photosynthesis at constant light intensity under continuous or nonfluctuating irradiation (Michio, 2018). This concept has been used in various studies with LEDs because they can blink or flash briefly, known as pulse lighting (Figure 1). This is achieved by rapidly turning on and off the lamp output at intervals of only a few microseconds (μs), which results in intensely focused intermittent light that uses less energy (Olvera-Gonzalez et al., 2021). The primary characteristics of pulsed light are frequency, light intensity, and duty cycle. Pulsed light is an intermittent source of light that can be produced periodically (Chen et al., 2023).

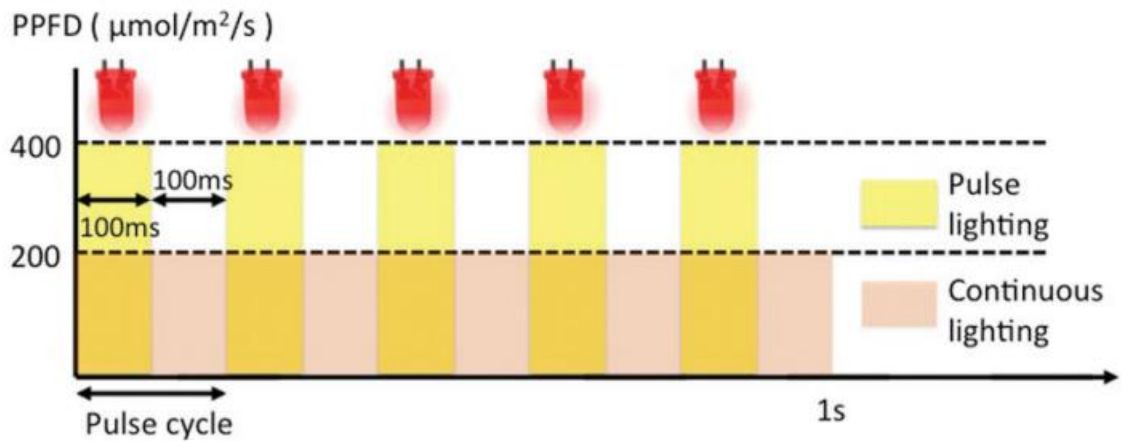


Figure 1. LED base pulse lighting under control growth environment(Michio, 2018).

Understanding how plants use the light they receive to optimise photosynthetic illumination is critical. Photons absorbed by photosynthetic pigments are converted into energy that powers the light processes involved in photosynthesis, is thermally dissipated, or is re-emitted as fluorescence by chlorophyll (van Iersel et al., 2016). Researchers began exploring how pulsed light affected photosynthesis in the first phase of the 20th century. Harun et al. (2013) found very significant results for plants with a pulse lighting control system of 1-hour duration with 15 minutes light-off period compared to a typical photoperiod at 12 hours continuous lighting system and 12 hours light period. The data analysis concluded that plants with a pulse light treatment recorded 291% higher photosynthesis rates and had better growth rates than conventional 12-hour lighting systems. The impact of pulsed light (0.5 and 1 kHz) on lettuce development showed that compared to continuous light treatments, the growth characteristics of lettuce exhibited up to 36%, 32%, and 48% greater values in terms of fresh biomass, leaf area and dry biomass under pulsed light (Miliauskiene et al., 2021). The improvement of photosynthesis under pulsed light from physiological mechanisms has been examined in several studies. For example, the quantity of adenosine triphosphate (ATP) produced in the electron transfer chain during the light phase is sufficient to sustain the Calvin cycle throughout the dark phase of high pulsed illumination frequencies. Because the pulsed light exposure is too brief to cause damage or the repeated dark intervals allow the damage to be repaired, which can increase the photosynthetic rate and the photodynamic damage is lessened (Chen et al., 2023).

Utilising pulsed LEDs is anticipated to minimise power usage without impacting plant development. Olvera-Gonzalez et al. (2021) used three criteria to build a mathematical model of the quantum efficiency of the photosystem II of chilli pepper plants and energy consumption to determine the effects of pulsed LED light versus continuous LED light. They experimented with different photosynthetic photon flux densities at 50, 110, and 180 μmol m^{-2} s^{-1}, frequencies at 100, 500, and 1000 Hz, and duty cycles of 40, 50, 60, 70, 80, and 90%. They found no significant statistical differences between the operation modes (continuous and pulsed LED light). Moreover, pulsed frequency and a duty cycle achieved substantial energy savings in every light intensity (Olvera-Gonzalez et al., 2021). Previous research has also established the effects of pulsed light of various frequencies and duty cycles of LED illumination systems on the growth of leaf lettuce and various other plants have been evaluated ((Miliauskiene et al., 2021),(Michio, 2018),(Cho et al., 2013),(Son et al., 2018),(Son et al., 2016),(Wittmann et al., 2023)). However, there is yet to be a clear answer as to what duty cycle or frequency results in the most significant energy saving or reduction in cost per kilogram. Therefore, this research will examine various duty cycles and frequencies from the literature to determine the most effective protocol for producing the lowest cost per kilogram of basil (*Ocimum basilicum L.*) plants.

2. Materials and methods

2.1. Plant materials and growth condition

Experiments were conducted in plant growth chambers (Adaptis, Conviron, Australia) under controlled environmental conditions at the University of Western Australia. Basil (*Ocimum basilicum*) seeds from Happy Valley Seeds (Riverstone, Australia) were sown in Rockwool cubes (25 mm × 25 mm× 30 mm), presoaked in water and placed in Saxon Mini Greenhouse (380 mm × 240 mm × 180 mm). Seedlings were germinated in growth chambers in darkness and subjected to continuous LED lighting and pulse lighting; the photosynthetic photon flux density (PPFD) was approximately 250 µmol m^{-2} s^{-1}. The environmental conditions were 27 ± 1 ˚C Day/night temperatures, 60% relative humidity and a 16 h photoperiod. After six days, a customised hydroponic nutrient solution from Power Gro was supplied to the custom hydroponic deep water culture (DWC) growing system (Figure 2.) with an average concentration of nutrients (mg L^{-1}). Six seedlings were placed in a hydroponic DWC growing system for each treatment and exposed to pulsed LEDs for four weeks under the same growth conditions.

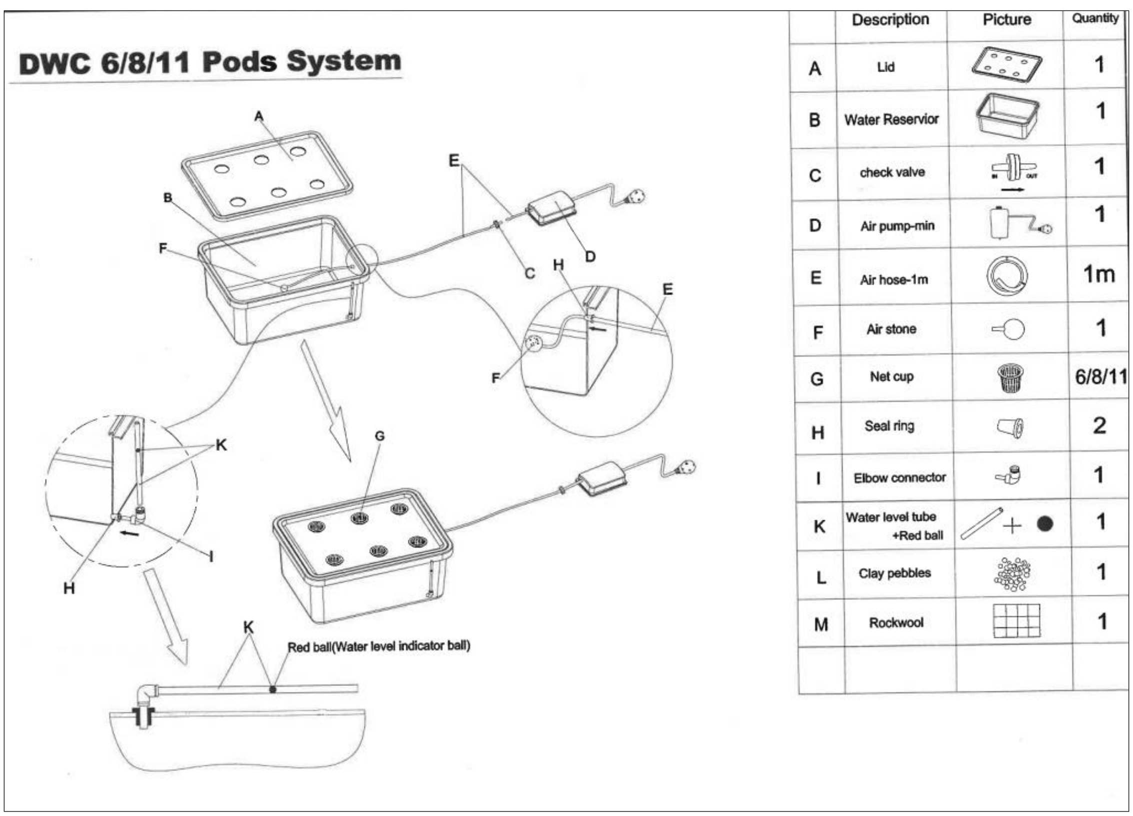


Figure 2. The custom Hydroponic Deep Water Culture (DWC) growing system

2.2. Pulse lighting treatments

Alex Montanari (AGRA Farming Technologies) developed the lighting systems for this research using the HLG 100 V2 LED Quantum Board (Horticultural Lighting Group, Westerville, USA). This design is a high-efficiency white light quantum board with 192 Samsung LM301H LEDs. This horticulture LED grow light produces ~16,000+ lumens with 95 watts of power (Figure 3).

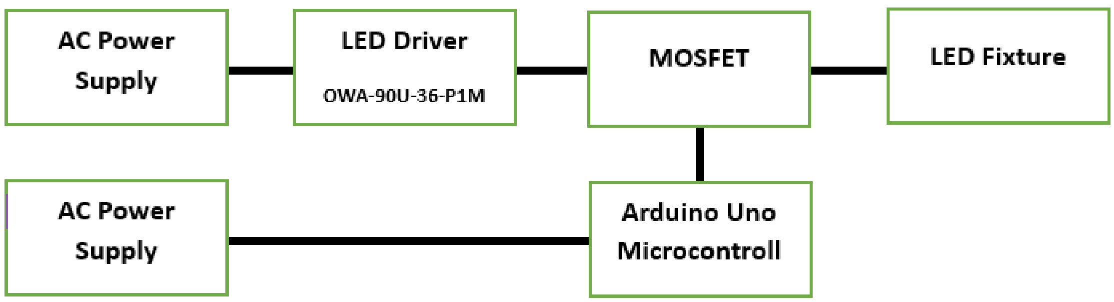


Figure 3. Block diagram of LED lighting system connected to Arduino Uno microcontroller.

The experiment was designed to compare continuous and pulse light effects on Basil plants. Six lighting treatments were designed with different duty cycles and frequencies (Table 1). Pulsed treatments of the LED lighting were operated using an Arduino Uno microcontroller connected to a MOSFET power switch module. The Arduino Uno controls the illumination using a code (Appendix 1.). Continuous light was used as the control for this research. After four weeks, the growth parameters of Basil plants, energy consumption, and energy use efficiency were measured.

Table 1. Experimental design

Light treatment	Duty cycle (%)	Frequency (Hz)
Continuous	100	-
A	85	50
B	75	1000
C	50	500
D	50	20,000

2.3. Basil shoots fresh weight and dry weight

The fresh and dry weights of shoots were measured four weeks after pulse LED lighting treatment. The weight of the shoots was measured on an electronic scale (Newton EJ-610 Portable Balance), after which the shoots were dried at 70˚C in an oven for three days to determine the dry weight.

2.4. Quantum yield of PSII (ΦPSII)

The proportion of light absorbed by chlorophyll at Photosystem II (PSII) and used for photochemistry at the current light intensity was measured after four weeks of onset LED treatment using a chlorophyll fluorescence meter (LI-600N; Licor LI-COR, Lincoln, USA). The third wholly developed leaf from the top was selected for these measurements.

2.5. Stomatal conductance to water vapour (G_{SW})

The capacity for water transport through the stomata of the third fully expanded basil leaf was measured after four weeks of growth using a chlorophyll fluorescence meter (LI-600N; Licor LI-COR, Lincoln, NE, USA). Measurements from both sides of the leaf were taken and summed to measure the total G_{SW}.

2.6. Light and energy use efficiency

Electric current and total energy consumption of plants under pulsed LED treatments and the control (throughout growth) were measured using a Fluke multimeter (FLUKE 115; Fluke, Everett, USA) and an electricity meter (Power-Mate Lite 10 Amp Power Meter, Australia). Energy use efficiency (EUE) was calculated by dividing shoot fresh weight by the PPFD (g FW·$PPFD^{-1}$), and total energy consumption ($W.g^{-1}$) was calculated by dividing power (W) by fresh weight (g) after four weeks of the experiment, respectively.

3. Results

Data for this research was collected through two experiments. The first experiment was unsuccessful due to unforeseen issues with the initial experimental setup, and data from the second experiment (physical observations of experiment two were attached to Appendix 4) became the focus of this research. However, a comparison between the first and second experiment data sets is attached as Appendix 2. The physical observations from Experiment 1 are presented in Figure 4, highlighting leaf abnormalities likely caused by pest attacks or micronutrient deficiencies.

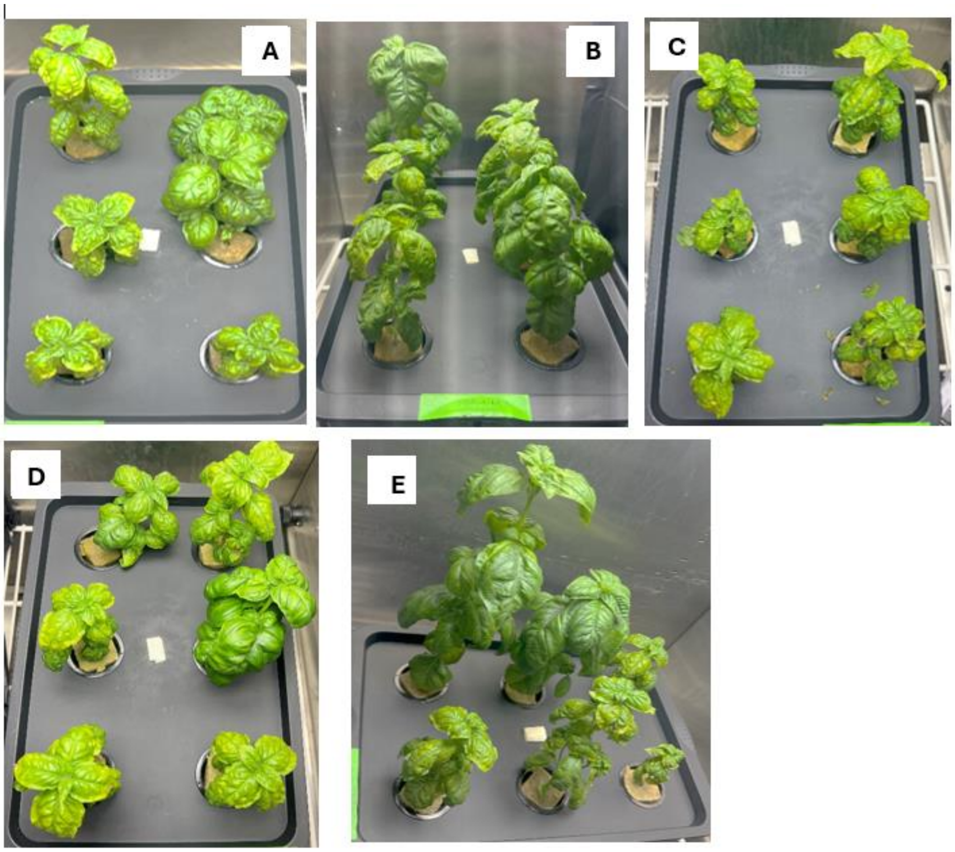


Figure 4. First experiment observations after 4 weeks of light treatment. Each letter indicates a light treatment: A – control, B- 85%-0.05khz, C-75%-1khz, D-50%-0.5khz and E-50%-20khz.

3.1. STOMATAL CONDUCTANCE

Stomata play a crucial role in balancing the plant's requirement for carbon dioxide uptake during photosynthesis with the need to minimise water loss through transpiration (Bonan, 2019). Stomatal conductance refers to the rate at which gases, including carbon dioxide and water vapour, pass through the stomata(Jones, 1998). Consequently, these results provide insights into the plant's transpiration rate, photosynthetic efficiency, and overall water use efficiency(Jones, 1998).

After four weeks of growth, the stomatal conductance of Basil plants was measured. The experiments were duplicated for each light treatment, utilising six basil plants as replicates per treatment. Individual lighting protocols were compared with the control treatment using one tail T-test with a 95% confidence level.

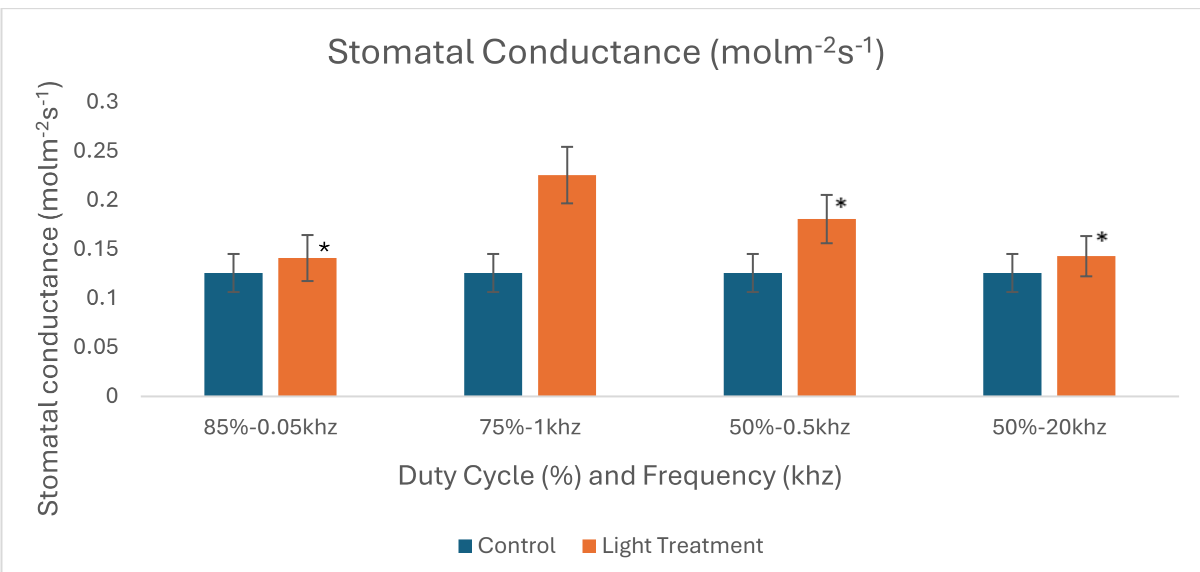


Figure 5. Effect of pulse lighting on stomatal conductance of Basil plants. The bar graph illustrates the impact of varying duty cycles and frequencies on the stomatal conductance of basil plants over 4 weeks. The duty cycles and frequencies include 85%-0.05khz, 75%-1khz, 50%-0.5khz, and 50%-20khz. The control group was represented in blue, while light treatments were depicted in orange. Statistical analysis included a one-tailed paired t-test with unequal variance for sample means (95% confidence level) and no significant difference indicated by an asterisk.

The varying duty cycle and frequency of LED lights have significantly affected the stomatal conductance of Basil plants compared to continuous light. Each treatment exhibited higher stomatal conductance compared to the control. The 75% duty cycle with 1kHz frequency resulted in the highest stomatal conductance value, while the 85% duty cycle with 0.05kHz frequency yielded the lowest stomatal conductance. However, the 85%-0.05 kHz treatment and the 50%-20 kHz treatment exhibited comparable values, which were closely aligned with those observed in the control group, and it shows that there is no statistically significant difference between the control and those two treatments. Thus, a significant difference was observed between the control and the other two light treatments (75%-1khz and 50%-0.5khz) at the 95% confidence level.

3.2 QUANTUM EFFICIENCY

Quantum yield constitutes a critical parameter in photochemistry and plant physiology, utilised to evaluate light energy conversion efficiency in plants (Baker, 2008). The findings provide significant insights into light use efficiency and the overall efficiency of photosynthesis and may serve as indicators of plant stress and damage(Baker, 2008).

Four pulse lighting treatments were compared with continuous light treatment to determine the effect on quantum yield (Figure 6). The results indicate no statistically significant difference between each pulse light treatment and control light treatment (P value > 0.05) except for the treatment 75%-1khz. Pulse light treatment with a 50% duty cycle and 0.5khz frequency has indicated the greatest quantum efficiency, while light treatment with a 75% duty cycle and 1khz frequency shows the least quantum efficiency. According to the literature, 0.83 is usually considered the optimum quantum efficiency (Son et al., 2018). However, the current results indicate that the quantum efficiency of Basil plants was lower than the optimum value. This reduction in efficiency may be attributed to stress induced as a side effect of pulse lighting.

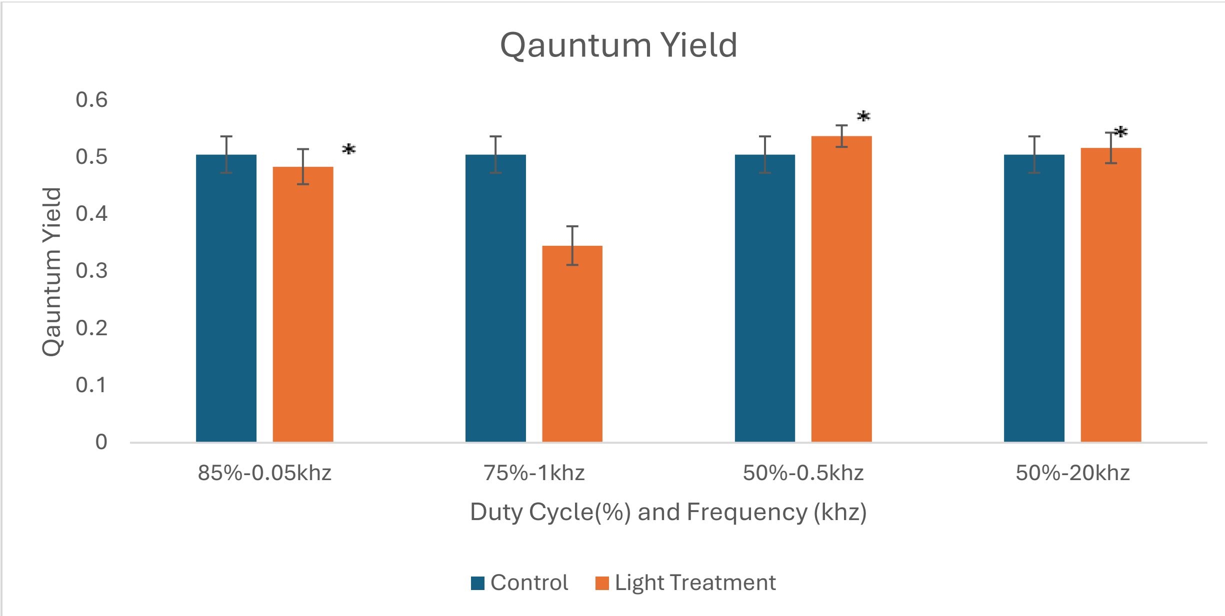


Figure 6. Impact of pulse lighting on the quantum yield of photosystem II in Basil plants. The bar graph illustrates the impact of varying duty cycles and frequencies on the quantum yield of PS II in basil plants over 4 weeks. The duty cycles and frequencies include 85%-0.05khz, 75%-1khz, 50%-0.5khz, and 50%-20khz. The control group was represented in blue, while light treatments were depicted in orange. Statistical analysis included a one-tailed paired t-test with unequal variance for sample means (95% confidence level) and no significant difference indicated by an asterisk.

3.3. GROWTH PARAMETERS

Optimising LED lighting can ensure optimal plant growth while lowering energy consumption. Figures 7 and 8 illustrate how duty cycle and frequency impact various growth parameters of basil plants. Fresh and dry weights from the pulse light treatments demonstrate higher values than the control treatment. These growth parameters show no significant differences between the control and other pulse light treatments. The light treatment with a 50% duty cycle and 20kHz frequency yielded slightly higher weight values in both fresh and dry weights. Conversely, the other three treatments have similar fresh and dry weight values. Overall, the fresh weight and dry weight trends suggest that the light treatments do not cause significant differences in fresh weight and dry weight compared to the control for most conditions, as indicated by the overlapping error bars.

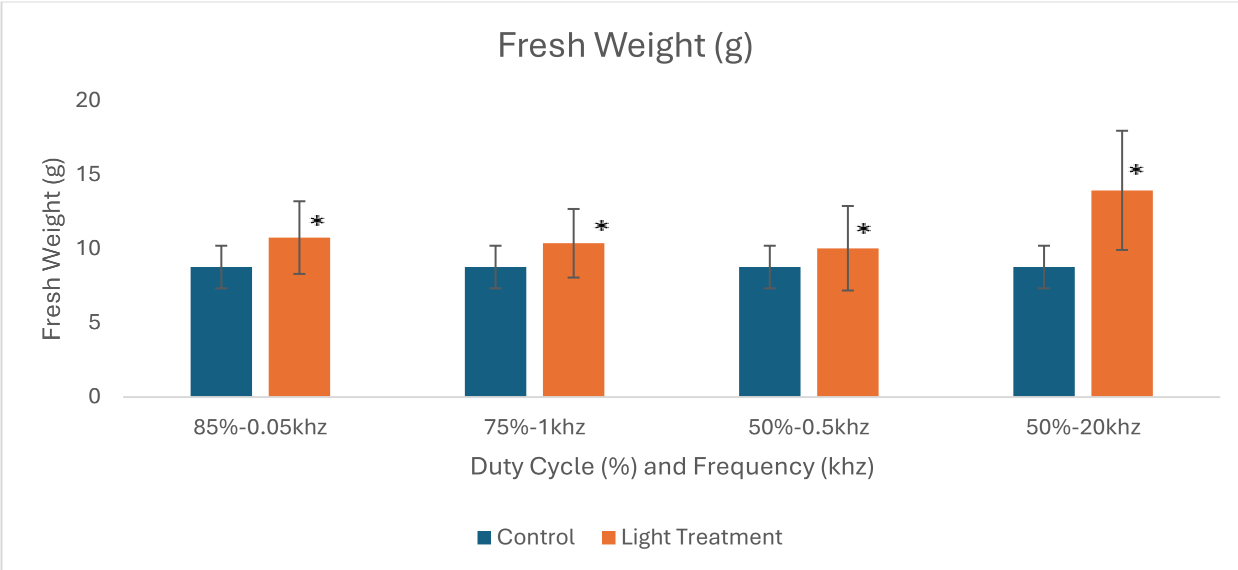


Figure 7. Effect of pulse lighting on fresh weight of Basil plants. The bar graph illustrates the impact of varying duty cycles and frequencies on the fresh weight of basil plants over a 4-week period. The duty cycles and frequencies include 85%-0.05khz, 75%-1khz, 50%-0.5khz, and 50%-20khz. The control group was represented in blue, while light treatments were depicted in orange. Statistical analysis included a one-tailed paired t-test with unequal variance for sample means (95% confidence level) and no significant difference indicated by an asterisk.

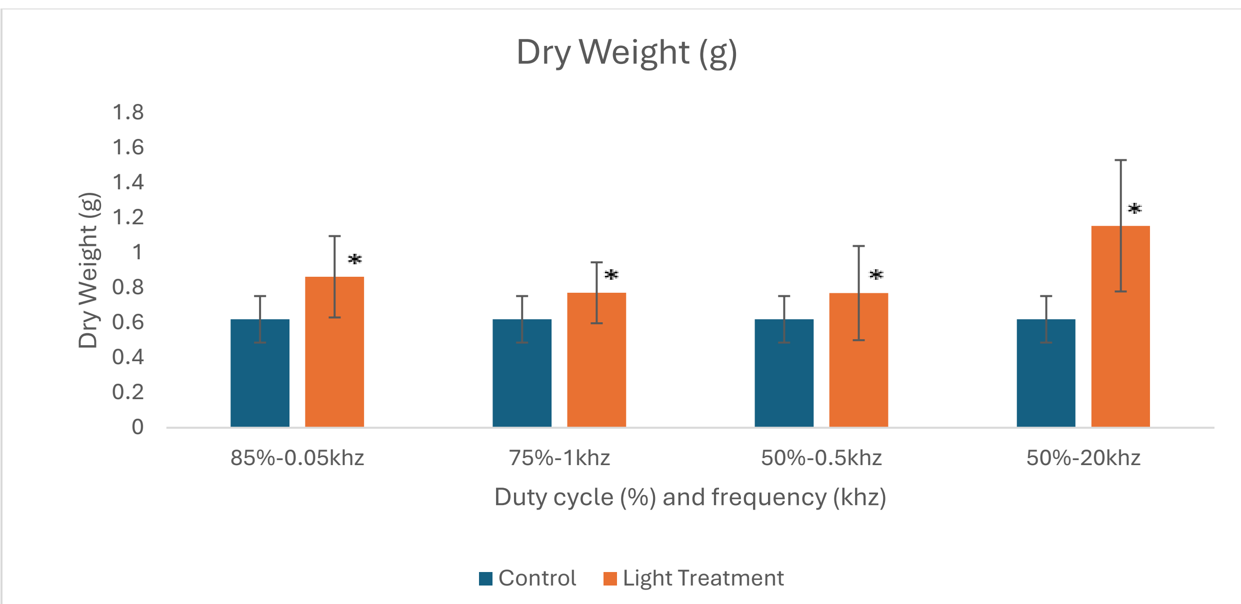


Figure 8. Impact of pulse lighting on dry weight of Basil plants. The bar graph illustrates the impact of varying duty cycles and frequencies on the dry weight of basil plants over a 4-week period. The duty cycles and frequencies include 85%-0.05khz, 75%-1khz, 50%-0.5khz, and 50%-20khz. The control group was represented in blue, while light treatments were depicted in orange. Statistical analysis included a one-tailed paired t-test with unequal variance for sample means (95% confidence level) and no significant difference indicated by an asterisk.

3.4. ENERGY CONSUMPTION EFFICIENCY

The energy consumption efficiency of pulse lighting was calculated using fresh weight and the power consumption used to grow basil plants (g/w; Figure 9.). All energy consumption values for pulse treatments were lower than those of the control, with the highest energy consumption observed in the 50%-0.5 kHz pulse light treatment, which also remained below the control value. This indicates an improvement in energy efficiency under pulse light treatments compared to continuous light treatment and demonstrates significant savings in energy cost per gram. Treatment at 50%- 20khz indicates the lowest cost per gram. However, as shown in Table 2, the pulse light frequency also significantly influences power consumption besides the duty cycle. Specifically, the results indicate that the power consumption for a duty cycle of 85% at 0.05 kHz and 50% at 20 kHz are comparable, suggesting that variations in frequency can offset the effects of different duty cycles on energy usage. Furthermore, no statistically significant difference exists between individual pulse treatments and the control.

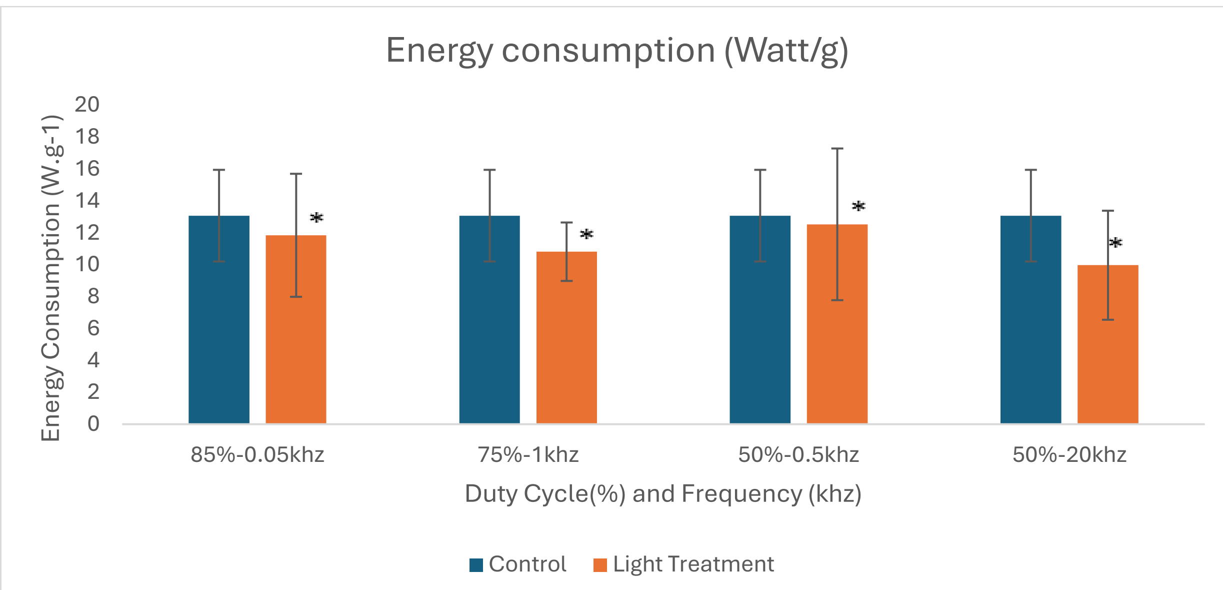


Figure 9. Impact of pulse lighting on energy consumption of Basil plants. The bar graph illustrates the impact of varying duty cycles and frequencies on the energy consumption of basil plants over a 4-week period. The duty cycles and frequencies include 85%-0.05khz, 75%-1khz, 50%-0.5khz, and 50%-20khz. The control group was represented in blue, while light treatments were depicted in orange. Statistical analysis included a one-tailed paired t-test with unequal variance for sample means (95% confidence level) and no significant difference indicated by an asterisk.

3.5. LIGHT USE EFFICIENCY

The basil plants' light use efficiency in different duty cycles and frequencies was calculated by dividing the fresh weight of basil plants by average PPFD, and PPFD was kept constant throughout the experiment (Figure 10.). Average PPFD was calculated by multiplying the control PPFD (250 μmol m^{-2} s^{-1}) by each corresponding duty cycle. According to the bar graph below, the light use efficiency of treatment "50%-20kHz" depicts the highest value while treatment "85%-0.05kHz" shows the lowest value. Additionally, there is an increasing trend in light use efficiency in pulse lighting compared to continuous light treatment. Moreover, no statistically significant difference exists between the control and pulse lighting treatments with 85% and 75% duty cycle (P value > 0.05). However, a significant difference exists between control and pulse light treatments with a 50% duty cycle.

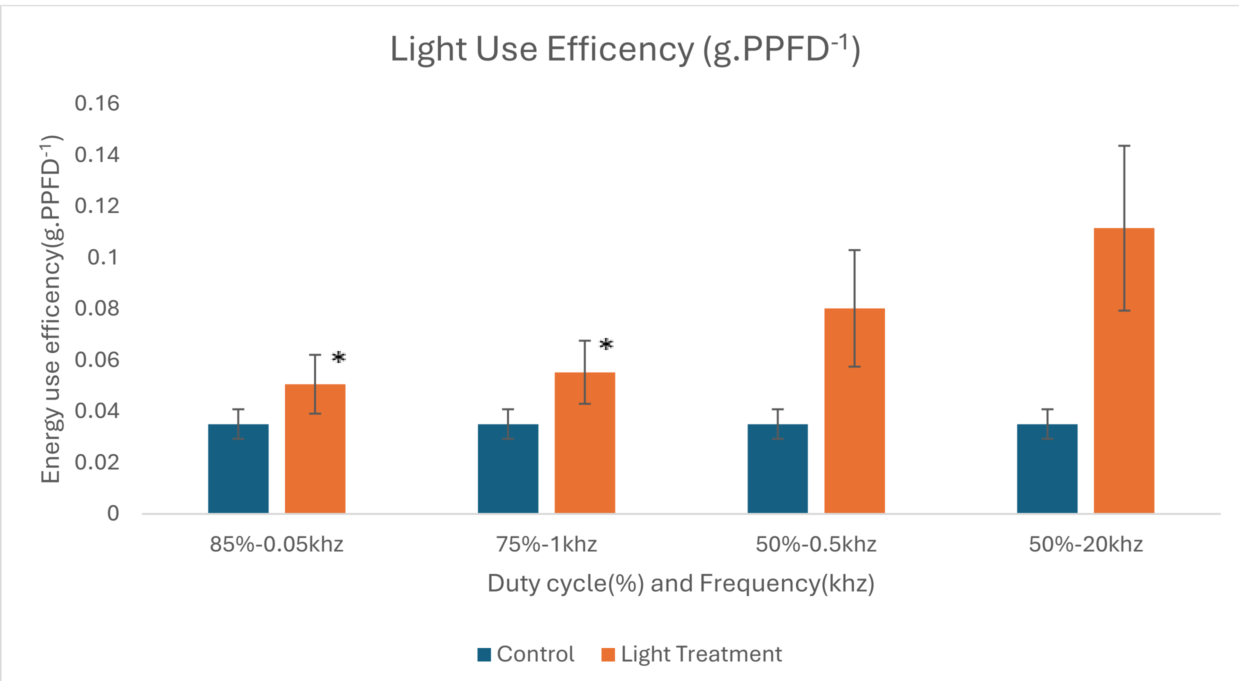


Figure 10. Impact of pulse lighting on light use efficiency of Basil plants. The bar graph illustrates the impact of varying duty cycles and frequencies on the light use efficiency of basil plants over a 4-week period. The duty cycles and frequencies include 85%-0.05khz, 75%-1khz, 50%-0.5khz, and 50%-20khz. The control group was represented in blue, while light treatments were depicted in orange. Statistical analysis included a one-tailed paired t-test with unequal variance for sample means (95% confidence level) and no significant difference indicated by an asterisk.

3.6 EFFECT DUTY CYCLE AND FREQUENCY TO POWER CONSUMPTION

A constant PPFD was maintained by adjusting the distance between the plant and the light source, and power consumption was quantified using an electricity meter. Although the PPFD was kept at a constant level, the overall light intensity received by the plants, averaged over the entire light and dark cycle, varies due to the influence of the duty cycle. This variation is reflected in Table 2 as the average PPFD, which was calculated by multiplying the control PPFD by each corresponding duty cycle. Additionally, Table 2 presents the power reduction for each treatment at steady PPFD. Treatment C, characterised by a 50% duty cycle and 500Hz frequency, exhibited the highest power reduction percentage, while Treatment B (75% duty cycle; 1kHz frequency) demonstrated the lowest power reduction percentage. Nevertheless, implementing a pulse width modulator to regulate the duty cycle and frequency of LED light significantly reduced power consumption.

Table 2. The percentage of reduction in power (W) at constant PPFD (250 µmol m^{-2} s^{-1}) for each pulse lighting treatment

Light treatment	Duty cycle (%)	Frequency (Hz)	Average PPFD	Power (W)	Power reduction (%)
Continuous	100	-	250	94.97	-
A	85	50	212.5	82.11	13.54
B	75	1000	187.5	92.31	2.80
C	50	500	125	69.99	26.30
D	50	20,000	125	83.28	12.30

4. Discussion

The feasibility of controlled environment agriculture is constrained by multiple factors, including the substantial costs incurred for energy utilised in light and temperature regulation. Integrating these technologies into agricultural practices is fundamentally linked to economic viability for growers, which is predominantly dependent on the minimisation of electricity expenditures(Song et al., 2019) . The exploration of pulsed LED lighting in vertical farming systems presents a compelling avenue for enhancing plant growth efficiency while mitigating energy expenditure. Our findings, aligned with the hypothesis that varying pulse frequencies and duty cycles can influence physiological plant responses, suggest that these lighting strategies may significantly affect basil plants' growth parameters and energy use efficiency and minimise cost per kilogram. Notably, the variation of pulse lighting parameters, such as duty cycle and frequency, as evidenced by our experimental results, plays a crucial role in optimising photosynthetic efficiency and growth outcomes.

Stomatal conductance:

Plants possess stomata that regulate CO_2 entry into the leaf and, consequently, substrate availability for photosynthesis. Furthermore, stomata are responsible for coordinating the intake of CO_2 for photosynthesis with the transpiration of water from the leaf (Slattery et al., 2017). When CO_2 input into the leaf is not required for photosynthesis, stomata can close to conserve water; however, stomata must open to supply the CO_2 necessary for the carbon reduction cycle (Slattery et al., 2017). Basil plants exposed to pulse light treatments in this study showed higher stomatal conductance than the control (Figure 5). However, the treatments at 85%-0.05 kHz and 50%-20 kHz exhibited stomatal conductance values comparable to the control, indicating that under these low lighting conditions, stomatal performance is similar to that observed in the control group. Consequently, it can be hypothesised that basil plants subjected to pulsed lighting should exhibit enhanced photosynthetic rates. This increase in photosynthetic activity is expected to correlate directly with an increase in biomass, as evidenced by a higher fresh weight in the treated plants compared to the control, and current experiment results justify this statement (Figures 7 and 8). Slattery et al. (2017) reported that, in conditions where water availability is not a constraint, an increased stomatal conductance in low-light environments is observable, and this phenomenon could mitigate thermal stress through evaporative cooling when plants are abruptly exposed to higher light intensities. However, it is important to recognise that this adaptation may decrease water use efficiency due to the higher transpiration rates associated with greater stomatal openings (Slattery et al., 2017). Therefore, optimising stomatal conductance in vertical farming significantly improves plant performance as it is a critical factor in photosynthesis, water use efficiency and overall plant health. Additionally, it has been demonstrated that, under pulse lighting conditions, plants perform similarly to those grown under continuous lighting conditions.

Quantum efficiency:

Three potential outcomes may occur upon absorption of light energy by chlorophyll molecules in a leaf: the energy can facilitate photosynthesis, be dissipated as heat, or be emitted as light through chlorophyll fluorescence. These three processes compete with one another such that the enhancement of one process diminishes the efficacy of the others (Maxwell & Johnson, 2000). These chlorophyll fluorescence measurements can be used to define quantum yield (van Iersel et al., 2016). The ΦPSII suggest the fraction of illumination absorbed by chlorophyll linked with Photosystem II and is consumed for the photochemistry of the plant. The higher the ΦPSII value, the greater the quantum efficiency of Photosystem II (Maxwell & Johnson, 2000). In the current study, figure 6. showed that the quantum yield of basil plants grown under pulse lighting and continuous lighting environment have comparable ΦPSII measurements except for treatment 75%-1khz. The overall experiment results also show a reduction in ΦPSII measurements compared to the optimum ΦPSII measurements. This reduction in Photosystem II (PSII) efficiency may have occurred due to either one of these primary processes or a combination of both. The first involves inactive PSII centers due to an overabundance of reduced electron acceptors (Demmig-Adams et al., 1996). This condition leads to energy breakdown within these centers, subsequently generating harmful reactive oxygen species. The second process involves reducing PSII's ability to effectively capture excitation energy when plants employ preemptive thermal dissipation mechanisms (Demmig-Adams et al., 1996). These mechanisms release excess energy as heat before it reaches the PSII centers, helping to prevent energy accumulation. This thermal regulation strategy aids in avoiding PSII overload and maintains the stability of the electron transport chain (Demmig-Adams et al., 1996). Furthermore, when comparing the 50% duty cycle results with low and high frequency treatments, the pulse light experiment with lower frequency had higher quantum yield than higher frequency. Olvera-Gonzalez *et al.* (2014) also observed similar results and stated that the plant lacks sufficient time to unload received energy at high frequencies characterised by shorter darkness periods. Conversely, plants have more time to process absorbed energy at low frequencies with extended dark periods. Given adequate time for the plant to dissipate energy from the light source, less energy will be emitted as heat, optimising radiation absorption and enhancing the photosynthetic processes (Olvera-Gonzalez et al., 2014). Moreover, studies by Olvera-Gonzalez et al. (2014), Son et al. (2018), and Michio (2018) reported similar findings to the current study, indicating that the operation of the photosynthetic system in plants remains normal under specific duty cycle and frequency conditions. Overall results suggest that while certain pulse light treatments can maintain quantum yield at levels comparable to continuous illumination, not all frequencies and duty cycles demonstrate equal efficacy. In vertical farming, selecting an appropriate light treatment is critical to ensure optimal photosynthetic efficiency without incurring unnecessary energy consumption.

Growth parameters:

The biomass accumulation of basil plants was studied in the present research. The growth parameters of basil plants, measured by fresh weight assimilation, showed increases of up to 22%, 18%, 14%, and 58% in the 85%-0.05 kHz, 75%-1 kHz, 50%-0.5 kHz, and 50%-20 kHz pulsed light treatments, respectively. Similarly, dry weight measurements indicated enhancements of 38%, 24%, 24%, and 85% under the same pulsed light conditions. Comparable findings were observed in the study by Miliauskiene et al. (2021), which examined lettuce growth under pulsed light treatments with a 50% duty cycle at 0.5 kHz and 1 kHz frequencies. The research reported that plants exposed to pulsed light showed a 36% enhancement in fresh weight and a 48% improvement in dry weight compared to those grown under continuous lighting conditions. Furthermore, Son et al. (2018) reported that the fresh weight of lettuce grown under pulsed lighting conditions (75% duty cycle at frequencies of 0.3, 1, 3, 10, and 30 kHz) showed no significant difference compared to the control treatment. Additionally, Michio (2018) reported that the fresh weight of lettuce shoots increased significantly, by up to 20%, at higher frequencies (20–1.3 kHz) compared to lower frequencies (500–0.5 Hz) under a 50% duty cycle, and also when compared to continuous light. These findings are consistent with the results of the current study for treatments utilising a 50% duty cycle, as shown in Figures 7 and 8. Therefore, our findings indicate that pulsed lighting can enhance fresh weight compared to continuous lighting. Among the pulsed lighting treatments, the 50% duty cycle at 20 kHz frequency yielded the highest increase in fresh weight.

Energy consumption (cost per gram) and light use efficiency:

The goal of using pulse lighting in this study is to reduce the cost of production by minimising energy consumption. As demonstrated in Figure 9, using pulsed illumination reduced energy consumption compared to the control treatment. Consequently, this reduction also led to a decrease in the cost per gram of basil biomass produced. According to the findings, treatment with a 50% duty cycle and 20khz frequency has the lowest cost per gram. Son et al. (2018), Cho et al. (2013), Son et al. (2016), Jao & Fang (2004), and Chen et al. (2023) found similar results for energy consumption in the current study (Jao & Fang, 2004). However, although power usage decreased with lower duty cycles, an increase in frequency was directed to higher power consumption. These findings are displayed in Table 2. We hypothesised that the increased power consumption associated with higher frequencies is directly related to the power driver. Therefore, although pulse lighting can reduce the cost per gram in vertical farming, a customised LED panel incorporating pulse lighting should be designed with an optimal duty cycle and frequency to achieve more accurate and efficient outcomes. Son et al. (2018) reported that although electrical energy consumption was lower under pulsed lighting, the PPFD was also comparatively lower than that of the control treatment in their study. In the present study, although we maintained a constant PPFD by adjusting the light source height, the average PPFD remained lower than that of the control treatment. These average PPFD measurements were subsequently used to construct the light use efficiency graph (Figure 10), and the result indicated that the light use efficiency for pulse lighting is higher than the continuous light use efficiency. Comparative findings regarding light use efficiency have been reported by Son et al. (2018), Cho et al. (2013), Son et al. (2016), Jao and Fang (2004), and Chen et al. (2023).

A comparison of stomatal conductance, quantum yield, and fresh weight data demonstrates that pulse lighting enhances light use efficiency compared to continuous light treatment. These findings further validate the effectiveness of pulse lighting, as reflected in the data.

Effect duty cycle and frequency to power consumption:

In pulsed lighting, the power consumption is proportional to the duty cycle, which represents the fraction of time the light is active. At a 50% duty cycle, for example, the light is on for half the time compared to continuous operation. This would lead us to expect roughly half the power consumption. This is because power is only drawn during each pulse's "on" phase, effectively reducing the average energy use over time.

When analysing the power consumption data for the different protocols, we discovered a disparity with this logic. For example, light treatment D (50%, 20kHz) only saw a 12.3% reduction in electricity compared to control, when we expected closer to 50%. This disparity continued with all protocols except light treatment A.

From this and further research, we determined that the LED driver (OWA-90U-36-P1M) was designed for continuous, steady-state current delivery, making it incompatible with the rapid switching in pulsed lighting. These drivers lack the response speed required to handle the frequent on-off cycles efficiently, resulting in improper current regulation and potentially higher power losses. This mismatch leads to a situation where power consumption does not correlate linearly with the duty cycle, as the driver was not optimized for this purpose.

As a result, we could not rely on the Watt/g metric to identify the most efficient protocols for use in Plant Factories. For commercial operations, reducing the cost per kilogram/gram ($/g) is the most important metric to improve the commercial viability of Plant Factories. Thus, the growing conditions/methodology that result in the lowest $/g are most favourable for commercial applications. With power consumption directly correlated to cost, Watt/g is directly proportional to $/g for lighting experiments.

Although the PPFD in the experiment was kept constant, the overall light intensity received by the plants, averaged over the entire light and dark cycle, varies due to the influence of the duty cycle (Figure 10). This shows that pulsed lighting can significantly reduce power consumption.

Following the expectation that power consumption is proportional to the duty cycle, and instead of reliable Watt/g measurements, Average PPFD/g provides the closest proxy to $/g and, thus, a representation of the most favourable protocol for commercial applications.

Conclusion

This study observed that the pulsed lighting treatment with a 50% duty cycle and 20 kHz frequency demonstrated the lowest energy use efficiency and the highest light use efficiency, which were crucial parameters for assessing the commercial viability of plant factories. However, regarding physiological responses, such as stomatal conductance and quantum yield, the highest values were recorded in the 75%-1 kHz and 50%-0.5 kHz treatments, respectively. Our findings indicate that pulsed lighting treatments significantly enhanced basil plant growth, as reflected by increased fresh and dry biomass while reducing power consumption compared to the control. Notably, power consumption increased with higher pulse frequencies. Therefore, further research must explore the relationship between power consumption and pulse light frequency. Although this study demonstrated that pulsed lighting improves basil yield, it is crucial to determine the optimal frequency and duty cycle for the most efficient use of light. We propose that implementing pulsed lighting in vertical farming could reduce the cost per gram of basil production without compromising growth.

5. References

Baker, N. R. (2008). Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo. *Annual review of plant biology*, *59*(1), 89-113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Bonan, G. (2019). Stomatal Conductance. In (pp. 189-212). Cambridge University Press. https://doi.org/10.1017/9781107339217.013

Chen, F., Zheng, J., Ma, H., Zhang, W., Fan, L., Zhang, F., Li, M., Omer, A. A. A., Zhang, X., & Liu, W. (2023). Pulsed-lighting LED luminaire for agriculture with a geometrical optical solution. *Optics express*, *31*(4), 5609-5624. https://doi.org/10.1364/OE.483071

Cho, K. J., Cho, J. Y., Park, I. S., & Oh, W. (2013). Effects of Duty Ratio of Pulsed LED Light on Growth and Photosynthetic Rate of Lettuce Grown in a Plant Factory System. *Journal of Korea Society for Plants People and Environment*, *16*(6), 427-434. https://doi.org/10.11628/ksppe.2013.16.6.427

Demmig-Adams, B., Adams Iii, W. W., Barker, D. H., Logan, B. A., Bowling, D. R., & Verhoeven, A. S. (1996). Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiologia Plantarum (København. 1948)*, *98*(2), 253-264. https://doi.org/10.1034/j.1399-3054.1996.980206.x

German, R. N., Thompson, C. E., & Benton, T. G. (2017). Relationships among multiple aspects of agriculture's environmental impact and productivity: a meta-analysis to guide sustainable agriculture. *Biological reviews of the Cambridge Philosophical Society*, *92*(2), 716-738. https://doi.org/10.1111/brv.12251

Jao, R.-C., & Fang, W. (2004). Effects of Frequency and Duty Ratio on the Growth of Potato Plantlets In Vitro Using Light-emitting Diodes. *HortScience*, *39*, 375-379. https://doi.org/10.21273/HORTSCI.39.2.375

Jones, H. G. (1998). Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany*, *49*(90001), 387-398. https://doi.org/10.1093/jexbot/49.suppl_1.387

Kozai, T., Niu, G., & Takagaki, M. (2019). *Plant Factory : An Indoor Vertical Farming System for Efficient Quality Food Production* (2nd ed.). Elsevier Science & Technology.

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany*, *51*(345), 659-668. https://doi.org/10.1093/jexbot/51.345.659

Michio, K. (2018). Growth and Photosynthesis under Pulsed Lighting. In C. Juan Cristóbal García & L. Gema Lorena López (Eds.), *Photosynthesis* (pp. Ch. 3). IntechOpen. https://doi.org/10.5772/intechopen.75519

Miliauskiene, J., Karlicek, R. F., & Kolmos, E. (2021). Effect of Multispectral Pulsed Light-Emitting Diodes on the Growth, Photosynthetic and Antioxidant Response of Baby Leaf Lettuce (Lactuca sativa L.). *Plants (Basel)*, *10*(4), 762. https://doi.org/10.3390/plants10040762

Olvera-Gonzalez, E., Alaniz-Lumbreras, D., Torres-Argüelles, V., González-Ramírez, E., Villa-Hernández, J., Araiza-Esquivel, M., Ivanov-Tsonchev, R., Olvera-Olvera, C., & Castaño, V. M. (2014). A LED-based smart illumination system for studying plant growth. *Lighting research & technology (London, England : 2001)*, *46*(2), 128-139. https://doi.org/10.1177/1477153513478300

Olvera-Gonzalez, E., Escalante-Garcia, N., Myers, D., Ampim, P., Obeng, E., Alaniz-Lumbreras, D., & Castano, V. (2021). Pulsed LED-Lighting as an Alternative Energy Savings

Technique for Vertical Farms and Plant Factories. *Energies (Basel)*, *14*(6), 1603. https://doi.org/10.3390/en14061603
Slattery, R. A., Walker, B. J., Weber, A. P. M., & Ort, D. R. (2017). The Impacts of Fluctuating Light on Crop Performance. *Plant Physiology*, *176*(2), 990-1003. https://doi.org/10.1104/pp.17.01234
Son, K.-H., Jeon, Y.-M., & Oh, M.-M. (2016). Application of supplementary white and pulsed light-emitting diodes to lettuce grown in a plant factory with artificial lighting. *Horticulture, environment and biotechnology*, *57*(6), 560-572. https://doi.org/10.1007/s13580-016-0068-y
Son, K. H., Lee, S. R., & Oh, M. M. (2018). Comparison of Lettuce Growth under Continuous and Pulsed Irradiation Using Light-Emitting Diodes. *Weon'ye gwahag gi'sulji*, *36*(4), 542-551. https://doi.org/10.12972/kjhst.20180054
Song, S., Kusuma, P., Carvalho, S. D., Li, Y., & Folta, K. M. (2019). Manipulation of seedling traits with pulsed light in closed controlled environments. *Environmental and experimental botany*, *166*, 103803. https://doi.org/10.1016/j.envexpbot.2019.103803
van Iersel, M. W., Weaver, G., Martin, M. T., Ferrarezi, R. S., Mattos, E., & Haidekker, M. (2016). A Chlorophyll Fluorescence-based Biofeedback System to Control Photosynthetic Lighting in Controlled Environment Agriculture. *Journal of the American Society for Horticultural Science*, *141*(2), 169-176. https://doi.org/10.21273/JASHS.141.2.169
Wang, Y., Tibbetts, S. M., & McGinn, P. J. (2021). Microalgae as sources of high-quality protein for human food and protein supplements. *Foods*, *10*(12), 3002. https://doi.org/10.3390/foods10123002
Wittmann, S., Jüttner, I., & Mempel, H. (2023). Pulsed light − optimal ratio between yield and energy reduction. *Acta horticulturae*(1369), 227-234. https://doi.org/10.17660/actahortic.2023.1369.28

APPENDIX 1: First and last page of the recent issue of the chosen journal: Plant Biotechnology Journal

Check for updates

Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2024), pp. 1–15 doi: 10.1111/pbi.14482

Flexible substrate-based mass spectrometry platform for *in situ* non-destructive molecular imaging of living plants

Guanhua Zhang[1,2], Han Zheng[2], Xiao Wang[1], Shuxin Han[1], Wei Liu[1], Chenglong Sun[1], Qiongzheng Hu[1] and Chunxia Ma[1,2,*]

[1]*Shandong Analysis and Test Center, Qilu University of Technology (Shandong Academy of Sciences), Jinan, China*
[2]*State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China*

Received 7 October 2023;
revised 23 August 2024;
accepted 2 September 2024.
*Correspondence (Tel +86 187 5316 2707; Fax +86 0531 82964889; email machunxia8927@163.com)

Keywords: Mass spectrometry imaging (MSI), Flexible substrate, Surface analysis, Living plant, Signalling molecules.

Summary

Monitoring and localizing molecules on living plants is critical for understanding their growth, development and disease. However, current techniques for molecular imaging of living plants often lack spatial information or require tedious pre-labelling. Here, we proposed a novel molecular imaging platform that combines sliver nanowire-doped Ti_3C_2 MXene (Ag NWs@MXene) flexible film substrate with laser desorption/ionization mass spectrometry imaging (AMF-LDI-MSI) to study the spatial distribution of biomolecules on the surface of living plants. This platform overcomes the MSI challenges posed by difficult-to-slice plant tissues (e.g., tough or water-rich roots and fragile flowers) and enables precisely transfer and visualize the molecule. Comparisons of the measurement results to those from matrix-assisted LDI-MSI (MALDI-MSI) technology demonstrate the accuracy and reliability of the platform. Biocompatibility evaluations indicated that the platform without observable adverse effects on the health of living plants. The distribution of growth and disease-associated signalling molecules, such as choline, organic acids and carbohydrates, can be in situ non-destructively detected on the surfaces of living plants, which is important for tracking the health of plants and their diseased areas. AMF-LDI-MSI platform can serve as a promising tool for label-free, in situ and non-destructive monitoring of functional biomolecules and plant growth from a spatial perspective.

Introduction

Plants are essential components of the biosphere and play crucial roles in preserving the stability and health of global ecosystems. Plant metabolites are integral to maintaining this process, helping plants grow and develop, respond to changing environments and resist pests. The region-specific accumulation of plant metabolites is a key factor influencing their growth, development and responses to abiotic and biotic environmental stresses (Fernie and Pichersky, 2015). Recent research has revealed that metabolic molecules present on the surface of plants can reflect their physiological status, stress stimulus processes and microbial communities (Dubey *et al.*, 2020; Shroff *et al.*, 2015). Monitoring signalling molecules on the surface of living plants is a promising way to understand physiological behaviour in the plant growth process; however, the effective detection of these molecules remains a long-standing challenge because of the relatively low concentrations and complex compositions of their active forms.

Modern metabolomic analytical techniques open the door to comprehensive profiling of plant phenotypes at the molecular level. Chromatographic and mass spectrometry techniques are used for the high-throughput analysis of target biomolecules (Qu *et al.*, 2022); however, their homogenization process with tissue damage prevents their widespread application for the simultaneous detection of metabolites in living plants. Most importantly, these techniques cannot be used to visualize the distribution of molecules on plant surfaces. Inspired by human wearable sensing devices, several emerging sensor platforms for plants have been proposed that can be attached to living plants for the continuous monitoring of their physiological characteristics. The application of these wearable sensors to plant science is still in its infancy and is usually restricted to monitoring the microclimates surrounding living organisms and single biomolecules such as H_2O_2, glutathione and phytohormone (Lew *et al.*, 2020; Li *et al.*, 2021; Son *et al.*, 2023). However, the composition of chemicals on the surfaces of living plants is multiple and varies over time, which poses great challenges for their precise identification and the determination of their location. Several advanced non-destructive monitoring techniques for living plants have been proposed to address the aforementioned difficulties, such as the nanoprobe technique based on surface-enhanced Raman scattering (Son *et al.*, 2023) and the Raman microscopy method (Yang *et al.*, 2017). However, these techniques cannot completely overcome the problems caused by the introduction of foreign impurities from nanomaterials or the interference of autofluorescence in plants. Therefore, a truly noninvasive molecular imaging method for the analysis of living plants with a wide dynamic range is urgently required.

Mass spectrometry imaging (MSI) is an emerging spatial metabolomics technology that analyses various compounds over a wide dynamic range with the advantages of *in situ* and

Please cite this article as: Zhang, G., Zheng, H., Wang, X., Han, S., Liu, W., Sun, C., Hu, Q. and Ma, C. (2024) Flexible substrate-based mass spectrometry platform for *in situ* non-destructive molecular imaging of living plants. *Plant Biotechnol. J.*, https://doi.org/10.1111/pbi.14482.

Q.H., Investigation and Supervision. C.M., Conceptualization, Resources, Supervision, Funding acquisition, Writing – review and editing.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Bai, S., Guo, X., Zhang, X., Zhao, X. and Yang, H. (2021) $Ti_3C_2T_x$ MXene-AgNW composite flexible transparent conductive films for EMI shielding. *Compos Part A-Appl S* **149**, 106545.

Bertin, C., Yang, X. and Weston, L.A. (2003) The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil* **256**, 67–83.

Chang, M., Liu, Y., Li, Z., Feng, X., Xiao, Y., Huang, W. and Liu, Y. (2024) Fingerprint analysis of volatile flavor compounds in twenty varieties of Lentinula edodes based on GC-IMS. *Sci. Hortic.* **328**, 112893.

Chen, W., Liu, L.X., Zhang, H.B. and Yu, Z.Z. (2020) Flexible, transparent, and conductive $Ti_3C_2T_x$ MXene-silver nanowire films with smart acoustic sensitivity for high-performance electromagnetic interference shielding. *ACS Nano* **14**, 16643–16653.

Coffinier, Y., Szunerits, S., Drobecq, H., Melnyk, O. and Boukherroub, R. (2012) Diamond nanowires for highly sensitive matrix-free mass spectrometry analysis of small molecules. *Nanoscale* **4**, 231–238.

Dubey, O., Dubey, S., Schnee, S., Glauser, G., Nawrath, C., Gindro, K. and Farmer, E.E. (2020) Plant surface metabolites as potent antifungal agents. *Plant Physiol. Biochem.* **150**, 39–48.

Dutkiewicz, E.P., Su, C.H., Lee, H.J., Hsu, C.C. and Yang, Y.L. (2021) Visualizing vinca alkaloids in the petal of *Catharanthus roseus* using functionalized titanium oxide nanowire substrate for surface-assisted laser desorption/ionization imaging mass spectrometry. *Plant J.* **105**, 1123–1133.

Fernie, A.R. and Pichersky, E. (2015) Focus issue on metabolism: metabolites, metabolites everywhere. *Plant Physiol.* **169**, 1421–1423.

Geng, D., Wang, R., Zhang, Y., Lu, H., Dong, H., Liu, W., Guo, L. *et al.* (2023) A 13-LOX participates in the biosynthesis of JAs and is related to the accumulation of baicalein and wogonin in *Scutellaria baicalensis*. *Front. Plant Sci.* **14**, 1204616.

Goodwin, R.J.A. (2012) Sample preparation for mass spectrometry imaging: small mistakes can lead to big consequences. *J. Proteomics* **75**, 4893–4911.

Harada, T., Yuba-Kubo, A., Sugiura, Y., Zaima, N., Hayasaka, T., Goto-Inoue, N., Wakui, M. *et al.* (2009) Visualization of volatile substances in different organelles with an atmospheric-pressure mass microscope. *Anal. Chem.* **81**, 9153–9157.

Harborne, J.B. and Williams, C.A. (2000) Advances in flavonoid research since 1992. *Phytochemistry* **55**, 487–504.

Iakab, S.A., Rafols, P., Tajes, M., Correig-Blanchar, X. and Garcia-Altares, M. (2020) Gold nanoparticle-assisted black silicon substrates for mass spectrometry imaging applications. *ACS Nano* **14**, 6785–6794.

Jiang, Y., Sun, J., Cui, Y., Liu, H., Zhang, X., Jiang, Y. and Nie, Z. (2019) Ti_3C_2 MXene as a novel substrate provides rapid differentiation and quantitation of glycan isomers with LDI-MS. *Chem. Commun. (Camb.)* **55**, 10619–10622.

Jiang, W., Lee, S., Zhao, K., Lee, K., Han, H., Oh, J., Lee, H. *et al.* (2022) Flexible and transparent electrode of hybrid $Ti_3C_2T_x$ MXene-silver nanowires for high-performance quantum dot light-emitting diodes. *ACS Nano* **16**, 9203–9213.

Jiao, X., Lu, X., Chen, A., Luo, Y., Hao, J. and Gao, W. (2015) Effects of *Fusarium solani* and *F. oxysporum* infection on the metabolism of Ginsenosides in American ginseng roots. *Molecules* **20**, 10535–10552.

Lee, G., Hossain, O., Jamalzadegan, S., Liu, Y., Wang, H., Saville, A.C., Shymanovich, T. *et al.* (2023) Abaxial leaf surface-mounted multimodal wearable sensor for continuous plant physiology monitoring. *Sci. Adv.* **9**, eade2232.

Lew, T.T.S., Koman, V.B., Silmore, K.S., Seo, J.S., Gordiichuk, P., Kwak, S.Y., Park, M. *et al.* (2020) Real-time detection of wound-induced H_2O_2 signalling waves in plants with optical nanosensors. *Nat Plants* **6**, 404–415.

Lew, T.T.S., Sarojam, R., Jang, I.C., Park, B.S., Naqvi, N.I., Wong, M.H., Singh, G.P. *et al.* (2020) Species-independent analytical tools for next-generation agriculture. *Nat Plants* **6**, 1408–1417.

Li, Z., Wang, L., Sun, D., Zhang, Y., Liu, B., Hu, Q. and Zhou, A. (2015) Synthesis and thermal stability of two-dimensional carbide MXene Ti_3C_2. *Mater Sci and Eng B* **191**, 33–40.

Li, R.J., Kuang, X.P., Wang, W.J., Wan, C.P. and Li, W.X. (2020) Comparison of chemical constitution and bioactivity among different parts of *Lonicera japonica* Thunb. *J. Sci. Food Agric.* **100**, 614–622.

Li, Z., Liu, Y., Hossain, O., Paul, R., Yao, S., Wu, S., Ristaino, J.B. *et al.* (2021) Real-time monitoring of plant stresses via chemiresistive profiling of leaf volatiles by a wearable sensor. *Matter* **4**, 2553–2570.

Li, L., Qiu, Z., Qi, Y., Zhao, D., Ali, I., Sun, C., Xu, L. *et al.* (2022) AuNPs/NiFe-LDHs-assisted laser desorption/ionization mass spectrometry for efficient analysis of metronidazole and its metabolites in water samples. *J. Hazard. Mater.* **423**(Pt A), 126893.

Li, X., Sun, R., Pan, J., Shi, Z., Lv, J., An, Z., He, Y. *et al.* (2023) All-MXene-printed RF resonators as wireless plant wearable sensors for in situ ethylene detection. *Small* **19**, e2207889.

Liu, X., Chen, Z., Wang, T., Jiang, X., Qu, X., Duan, W., Xi, F. *et al.* (2022) Tissue imprinting on 2d nanoflakes-capped silicon nanowires for lipidomic mass spectrometry imaging and cancer diagnosis. *ACS Nano* **16**, 6916–6928.

Liu, S., Dong, H., Zhang, M., Geng, W. and Wang, X. (2024) Identification of different degrees of processed ginger using GC-IMS combined with machine learning. *J. Pharm. Anal.* **14**, 149–151.

Lu, S., Sturtevant, D., Aziz, M., Jin, C., Li, Q., Chapman, K.D. and Guo, L. (2018) Spatial analysis of lipid metabolites and expressed genes reveals tissue-specific heterogeneity of lipid metabolism in high-and low-oil *Brassica napus* L. seeds. *Plant J.* **94**, 915–932.

Ma, Z., Kang, S., Ma, J., Shao, L., Zhang, Y., Liu, C., Wei, A. *et al.* (2020) Ultraflexible and mechanically strong double-layered aramid nanofiber-$Ti_3C_2T_x$ MXene/silver nanowire nanocomposite papers for high-performance electromagnetic interference shielding. *ACS Nano* **14**, 8368–8382.

Ma, C., Wang, X., Zhang, H., Liu, W., Wang, D., Liu, F., Lu, H. *et al.* (2022) High-throughput screening and spatial profiling of low-mass pesticides using a novel Ti_3C_2 MXene nanowire (TMN) as MALDI MS matrix. *Chemosphere* **286**(Pt 2), 131826.

Nishidono, Y.S.A., Nishizawa, M., Fujita, T., Nakamoto, M. and Tanaka, K. (2018) Identification of the chemical constituents in ginger(*Zingiber officinale*) responsible for thermogenesis. *Nat. Prod. Commun.* **13**, 13 (7).

Qin, R., Li, P., Du, M., Ma, L., Huang, Y., Yin, Z., Zhang, Y. *et al.* (2021) Spatiotemporal visualization of insecticides and fungicides within fruits and vegetables using gold nanoparticle-immersed paper imprinting mass spectrometry imaging. *Nanomaterials (Basel)* **11**, 1327.

Qu, X., Hu, S., Li, T., Zhang, J., Wang, B. and Liu, C. (2022) Metabolomics analysis reveals the differences between *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd. *Front. Plant Sci.* **13**, 933849.

Qu, Y., Li, X., Wang, X. and Dai, H. (2022) Multifunctional AgNWs@MXene/AgNFs electromagnetic shielding composites for flexible and highly integrated advanced electronics. *Compos Sci Technol* **230**, 109753.

Samarah, L.Z., Tran, T.H., Stacey, G. and Vertes, A. (2021) Mass spectrometry imaging of bio-oligomer polydispersity in plant tissues by laser desorption ionization from silicon nanopost arrays. *Angew. Chem. Int. Ed. Engl.* **60**, 9071 9077.

Shaposhnikov, A.I., Shakhnazarova, V.Y., Vishnevskaya, N.A., Borodina, E.V., Lebedinskii, M.I. and Strunnikova, O.K. (2024) Root exudation of barley with different resistance to root rot caused by *Fusarium culmorum*. *Russ. J. Plant Physiol.* **70**, 212.

Shimma, S. and Sagawa, T. (2019) Microscopy and mass spectrometry imaging reveals the distributions of curcumin species in dried turmeric root. *J. Agric. Food Chem.* **67**, 9652–9657.

Shroff, R., Schramm, K., Jeschke, V., Nemes, P., Vertes, A., Gershenzon, J. and Svatoš, A. (2015) Quantification of plant surface metabolites by matrix-assisted laser desorption-ionization mass spectrometry imaging: glucosinolates on *Arabidopsis thaliana* leaves. *Plant J.* **81**, 961–972.

APPENDIX 2. Code for Pulse lighting control

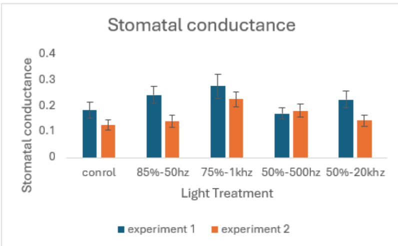
PWM Pulse Lighting Control

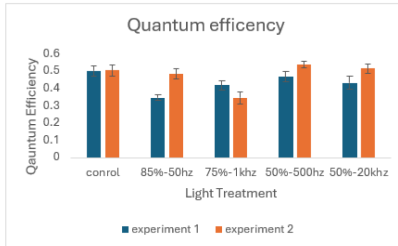
Alex Montanari

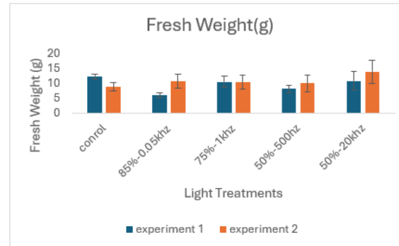
```
#include <PWM.h>
#include <Arduino.h>


const int LED = 9;

// Set PWM frequency to 500Hz (approximately)
int32_t frequency = 500;

void setup() {
  Serial.begin(115200);

  InitTimersSafe();

  SetPinFrequencySafe(LED, frequency);

  pinMode(LED, OUTPUT); // Set LED pin as an output

}

void loop() {
  // Get the current time
  unsigned long currentMillis = millis();
  int currentHour = (currentMillis / (1000 * 60 * 60)) % 24; // Convert milliseconds to hours

  /*
   *                          Automations
   */

  /* ///////////////////////////////////////////////////////////
   *  ////////// Pulsed Lighting Automation /////////////////
  ///////////////////////////////////////////////////////////////*/

  // If it's between midnight (0) and 4 PM (16), turn on the LED
  if (currentHour >= 0 && currentHour < 16) {
    pwmWrite(LED, 127); // 50% duty cycle (0-255 range)
    delay(50);
  } else {
    // Otherwise, turn off the LED
    pwmWrite(LED, 0);
    delay(50);
  }
}
```

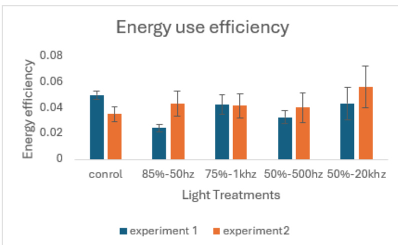
APPENDIX 3

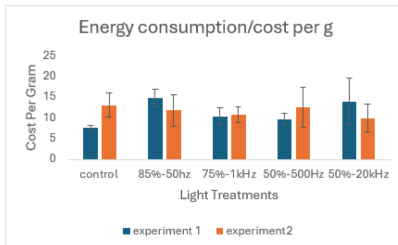
Comparison of Experiment 1 and 2 data analysis











APPENDIX 4 –

Experiment 2 physical observations

Basil plants after 4 weeks of light treatment. Each letter indicates a light treatment: A – control, B- 85%-0.05khz, C-75%-1khz, D-50%-0.5khz and E-50%-20khz.

A

B

C

D

E