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# Growth and Photosynthesis under Pulsed Lighting

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## Abstract

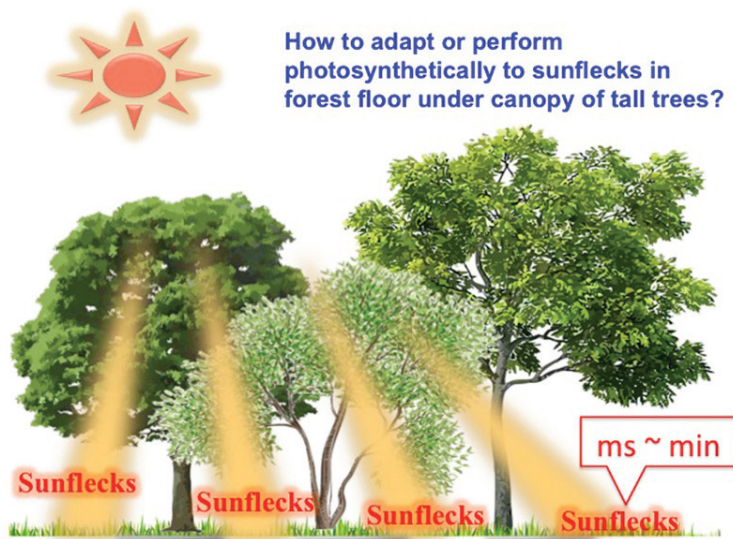
The effects of pulsed irradiation based-LEDs on the growth and photosynthetic light utilization efficiency of lettuce leaves were studied. Plants were grown under different pulse-cycled irradiations of 0.5–500 Hz, and 1–20 kHz frequencies, at PPFD of 200  $\mu\text{molm}^{-2} \text{s}^{-1}$  with 50% duty ratio (illuminated duration/cycle). The photosynthetic rate (Pn) was maintained relatively constant over the range of measurements at pulsed light at 80 PPFD. At 200 PPFD, Pn gradually decreased by lowering frequency below 2.5 Hz of pulsed light. Pn under pulsed light was slightly higher than that under continuous light. Chlorophyll fluorescence (Fv/Fm, Fv'/Fm', qP) showed no significant difference between under pulsed light and continuous light except at the lowest frequency (0.2 Hz). The similar quantum yield ( $\Phi\text{PSII}$ ) and electron transport rate (ETR) of PSII were obtained in a wide range of frequency of pulsed light, which might be an effective illumination strategy for cultivating leaf lettuce by using LEDs. Flashing irradiation did not significantly change chlorophyll content. Results suggested the effectiveness of pulsed light at 50% duty ratio on the growth of leafy vegetables that were richly cultivated in a closed type plant factory with the possibility of saving electricity by using intermittent illumination system with LEDs.

**Keywords:** chlorophyll fluorescence, CO<sub>2</sub> uptake rate, frequency, lightfleck, quantum yield

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## 1. Introduction

Plants or leaves in their natural state are frequently subjected to large and rapid fluctuations in irradiance. Photosynthetic performance under fluctuating irradiation (pulsed light or irregular sunflecks in forest floor, **Figure 1**) is different from steady-state photosynthesis under continuous or nonfluctuating irradiation at constant light intensity [1]. For example, poplar leaves receive 15% of their light in flecks lasting between 0 and 200 ms and a further 35% in flecks



**Figure 1.** Pulsed light or irregular sunflecks in forest floor.

between 200 and 400 ms [2]. Photosynthesis consists of light reaction and dark reaction as a continuous reaction in this order. The former, which is a chain redox reaction of photosystem II (PSII) and I (PSI), works as light energy harvesting and producing utilizable chemical energy products in the later  $\text{CO}_2$  assimilate reaction cycle, which reacts only enzymatically and light independently if adequate amount of these chemical energy products are supplied. The complex web of reactions in photosynthesis have different response times, so that fluxes through some reactions can be much faster than others resulting in fluctuating pool sizes. Furthermore, each reaction process seems to occur very rapidly in nanosecond to millisecond rates in the light reaction [3] as compared to seconds to minute rates in the dark reaction [4]. In recent years, photosynthetic responses to intermittent irradiation have been investigated again by using a developed illumination system with light-emitting diodes (LEDs). Measurement techniques also have a range in response times so that different reactions can be monitored with different temporal resolution.

Most of plant factory systems for producing leafy vegetables have adopted tubular cool-white fluorescent lamps as their light source. Recently, advances in LEDs technology have contributed to grow plants as a new type of light source. For further developing and improving plant factory system, LEDs illumination systems provide a potential alternative to the tubular fluorescent lamps due to their lower energy consumption, wavelength specificity and supposed durability.

Plant growth and development are strongly affected by light intensity (PPFD), quality (wavelength), and duration (photoperiod). The photosynthetic system including chlorophyll content, stomata size and leaf area of lettuce leaves was optimized by adjusting the light spectrum (455, 660, 735 nm) and flux density with high-power LEDs [5]. Light quality was

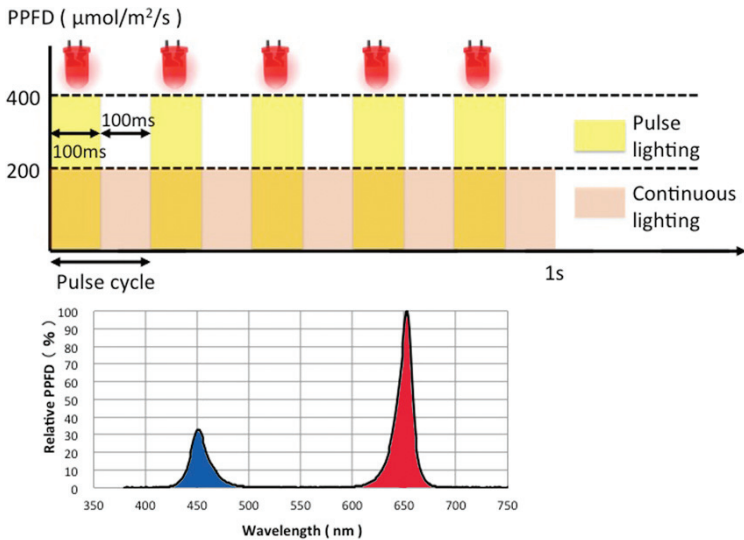
found critical not only to growth but also to biosynthesis of secondary metabolites in lettuce plants and especially the supplemental irradiation of green LEDs with the combination of red and blue LEDs can improve the growth [6]. Radiation mixture of blue, red, or far-red light with LEDs improved vegetable growth and enhanced the number of floral buds of ornamentals under controlled environmental conditions [7].

LEDs illumination system can blink or flash with a very short period, in which they can be turned fully on and fully off extremely rapidly ( $\mu\text{s}$  interval), emitting pulsed light with high intensity. Pulse light by adjusting the frequency and duty ratio (light on period per frequency) of LEDs resulted in optimal growth of potato plantlets *in vitro* with electricity savings and an effective illumination system adjusting light intensity, quality, frequency and duty ratio was developed [8–10]. In the pulsed light technique for growing tomato plants, low frequencies (0.1, 1, 10 Hz) had higher quantum efficiency in PSII than higher frequencies (50, 100 kHz) and continuous light, but the electron transport rate decreased when the frequency of pulse increased [11]. On the other hand, pulsed light of lower duty ratios, combined with lower frequencies, makes the  $\text{CO}_2$  uptake rate of cos lettuce lower than that attained in continuous light, inferring that pulsed illumination with such a condition is less advantageous than continuous light for photosynthesis [12].

The objectives of this study were to determine the effects of pulsed light with various frequencies of LEDs illumination system on the growth of leaf lettuce under controlled environmental conditions (PPFD,  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), and to investigate the leaf photosynthetic responses to pulsed light in comparison with continuous light (PPFD,  $0\text{--}500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The study was aimed to provide valuable information regarding the possibility of electricity savings in running plant factory system.

## 2. Measurements of $\text{CO}_2$ assimilation rates and chlorophyll fluorescence of intact leaves

Leaf lettuce (*Lactuca sativa* L. *crispa* 'Bio Saradana', Nakahara Seed Product Co., Ltd., Fukuoka, Japan) seeds were sown on watered sponge blocks ( $10 \times 10 \times 20 \text{ mm}$ ). After germination, 20 seedlings that had grown uniformly with three leaves were each transplanted into a polyvinyl pot (90 mm diameter, 80 mm depth) filled with vermiculite and grown for 30 days by bottom irrigation with commercial liquid fertilizer (OAT Agrio Co., Ltd., Tokyo, Japan, EC  $1.3 \text{ mS cm}^{-1}$ , pH 6.0). The air temperature and relative humidity throughout the cultivation were maintained at  $22^\circ\text{C}$  and 60%, respectively. The photosynthetic photon flux density (PPFD) was  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  supplied by LEDs lamps (Legu LED, HRD Co., Ltd. Tootori, Japan) providing a peak wavelength of red (660 nm) and blue (455 nm) with a 16-h day length (Figure 2). Fourteen irradiation treatments were examined to determine the effect of wide-range frequencies of pulsed lighting (20, 10, 4, 2, 1.3, 1 kHz, and 500, 50, 5, 2.5, 1.3, 1, 0.5 Hz at 50% duty ratio) and continuous lighting. After measurements of leaf photosynthetic parameters at the end of culture period, all plants were sampled and fresh weights of leaves and roots and total leaf area were recorded.



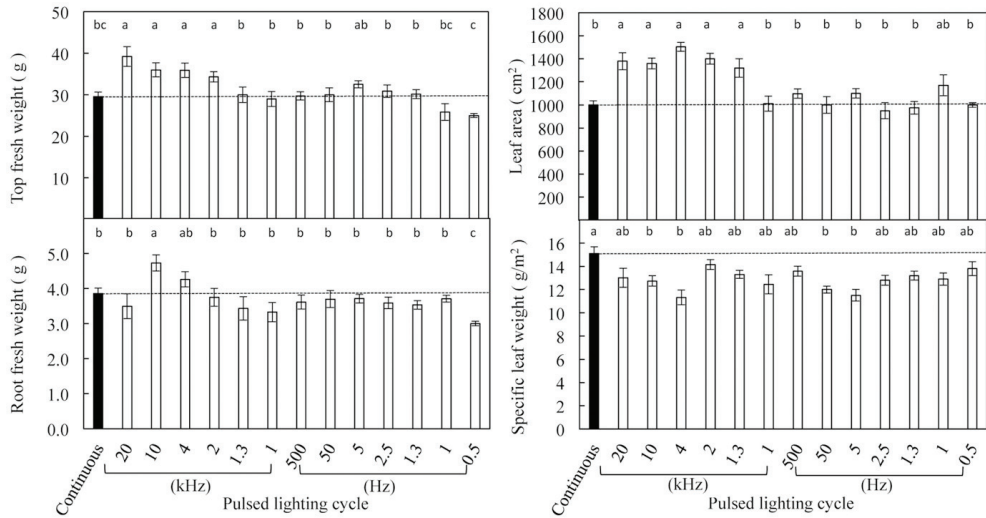
**Figure 2.** Pulsed lighting by using LEDs under growth conditions. Flashing pulse cycles were 20, 10, 4, 2, 1.3, 1 kHz, and 500, 50, 5, 2.5, 1.3, 1, 0.5 Hz, respectively, with 50% duty ratio (light on period: light off period = 1:1) and 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  effective PPFD at the top canopy of plants. As an example, 5 Hz pulsed or continuous lighting scheme during 1 s is shown in **Figure 2a** by using a LEDs lighting system which irradiates two narrow peak wavelengths of red (660 nm) and blue (455 nm) with 4:1 of light intensity (**Figure 2b**).

The  $\text{CO}_2$  uptake rates of a fully expanded mature single attached leaf were measured under varied PPFD and pulsed light conditions supplied by a same type of red-blue LEDs light source used for growing plants at constant leaf temperature, 22°C; leaf vapor pressure deficit, 1.0 kPa; and ambient  $\text{CO}_2$  concentration, 400  $\mu\text{mol mol}^{-1}$  using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). The apparent quantum yield ( $\Phi$ ) was estimated as a parameter of the best-fitted non-rectangular hyperbola for the photosynthetic responses to PPFD. Chlorophyll fluorescence has a more rapid response than  $\text{CO}_2$  uptakes, which enable photochemical and non-photochemical quenching to be measured during each flash. This can reveal the extent to which the transthylakoid  $\Delta\text{pH}$  gradient builds up with irradiance in continuous and flashing light. For each light treatment, parameters of chlorophyll fluorescence from a fully expanded mature single attached leaf were measured by using a FluorPen FP 100 (Photon Systems Instruments, Drasov, Czech Republic): maximum quantum yield ( $F_v/F_m$ ) in a dark-adapted leaf, effective quantum yield ( $F_v'/F_m'$ ) in an actinic light-adapted leaf with each pulse cycle.

### 3. Plant growth under pulsed light

The growth of lettuce leaves was affected by pulsed illumination as light source for hydroponic cultivation under controlled environmental conditions (**Figures 3, 4**). Shoot fresh weight

and total leaf area increased significantly by up to 20% at high frequencies (20–1.3 kHz) compared to low frequencies (500–0.5 Hz) and compared to continuous illumination. Meanwhile, pulse illumination had little effect on root growth because of its underground development without direct exposure to pulsed light. Leaf thickness was thinner under pulsed light, but the dependence on the frequency was not consistent. The growth estimated by total leaf area per plant of *Arabidopsis thaliana* was previously reported to increase by pulsed light with a frequency at 2.5 kHz and 45% duty cycle compared to continuous light under an average intensity of  $20 \mu\text{mol m}^{-2} \text{s}^{-1}$  supplied by LEDs [13]. It was explained that the growth increased



**Figure 3.** Growth characteristics (top and root fresh weights, total leaf area and specific leaf weight) of plants grown under continuous lighting (black column) and different pulsed lighting (empty columns) conditions at  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 20 days. Different lowercase letters represent significant differences ( $P \leq 0.05$ ) among lighting treatments and bars on column represent  $\pm \text{SE}$  ( $n = 10$ ).

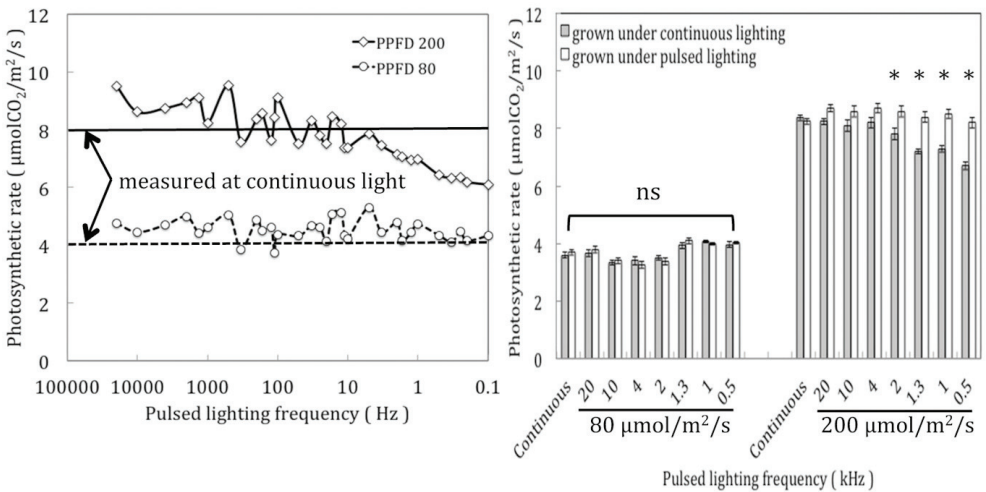


**Figure 4.** Leaf lettuce hydroponically grown under different pulsed lighting for 20 days.

at higher maximum light intensity of pulsed light than that of continuous light. The growth of lettuce increased by 23% at a pulse frequency of 2.5 kHz (50% duty ratio) under an average intensity of  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$  supplied by LEDs [14].

#### 4. Photosynthetic capacity measured at pulsed light

The photosynthetic rates (Pn) of mature leaves grown under continuous light were measured at pulsed light with various frequencies and at continuous light with two intensities (80 and  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). There were no significant differences between pulsed light with high frequencies (20 kHz–50 Hz) and continuous light, but Pn measured at low frequencies (2.5–0.1 Hz) gradually decreased to 75% of Pn measured at continuous light. Pn showed no difference between pulse and continuous measuring light when it was measured at low light intensity ( $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Figure 5). Pn measured at  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$  showed no difference between leaves grown under pulsed light and leaves grown under continuous light, but leaves grown under pulsed light showed no decrease in Pn at  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ . On the other hand, leaves grown under continuous light significantly decreased compared to pulse-irradiated leaves at lower frequencies than 2 Hz. Declined rates of Pn might be due to low scattering light in thicker leaves grown under continuous light compared than leaves grown under pulsed light as shown in Figure 3. For Pn-PPFD response curve (Figure 6) measured with leaves unfolded under continuous light, light-saturated Pn decreased by lowering pulse frequency of measuring light, resulting in low light saturation. The apparent quantum yield ( $\Phi$ )



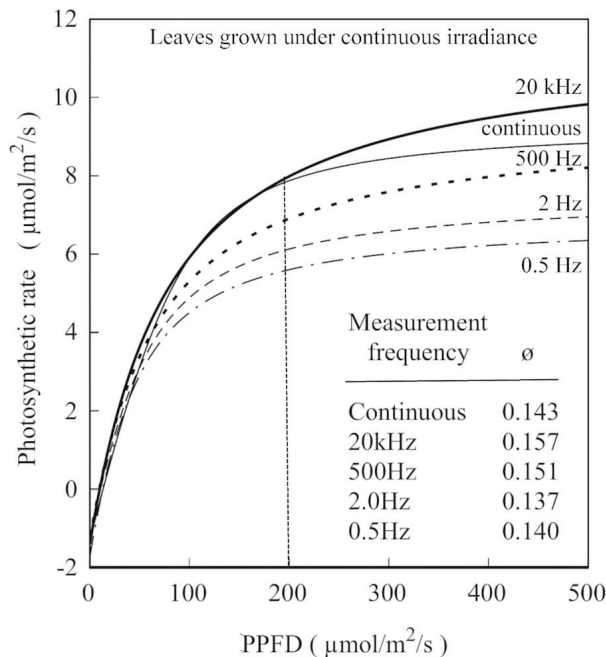
**Figure 5.** Photosynthetic rates of leaves grown under continuous light (left, broken curve) and under continuous or pulsed light (right, bar graph). PPFD during growing period was  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  with or without pulsed lighting. Measurement conditions of photosynthesis were 80 and  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD with continuous and with different frequencies of pulse lighting under  $400 \mu\text{mol mol}^{-1} \text{CO}_2$ . \* and ns show the significant and non-significant differences between two growth conditions.



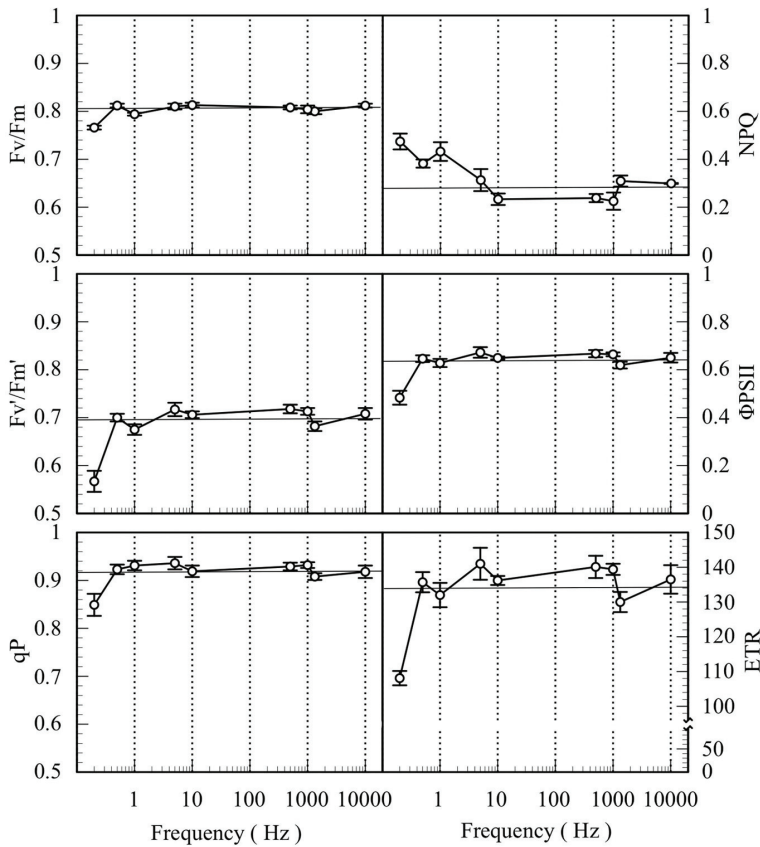
seemed to be higher at high frequency of pulsed measuring light. The quantum yield is well understood, and the maximum rate can be related to the electron transport capacity of the leaf. It may still influence the Pn-PPFD response via the transthylakoid  $\Delta pH$  gradient, which slows down electron transport by restricting plastoquinone reoxidation.

The maximum PSII quantum yield ( $F_v/F_m$ ) showed no difference in a wide range of pulse frequency (10 kHz–0.5 Hz) except for a significant decrease at 0.2 Hz (**Figure 7**), indicating that the function of PSII component might be affected by exposing leaves to slow flash light during their development. The effective PSII quantum yield induced in pulse light ( $F_v'/F_m'$ ) also decreased at only 0.2 Hz, indicating that there is no relationship between  $F_v'/F_m'$  and high frequency of pulsed light over 0.5 Hz. Similar tendencies were shown in  $qP$  and in calculated parameters ( $\Phi PSII$ , ETR). Decreases in these parameters indicate lowered efficiency of light energy utilization by the plant grown under pulsed light at low frequency (0.2 Hz). On the other hand, NPQ, which relates the distribution of light energy into non-photochemical processes by heat dissipation, increased at low frequencies below 1 Hz compared to NPQ measured at high frequencies above 5 Hz and at continuous light (**Figure 8**).

In leaves of tomato plants grown under pulsed light based-LEDs at a wide range of frequencies (100 kHz–0.1 Hz) with 50% duty cycle, the frequencies had both positive and negative



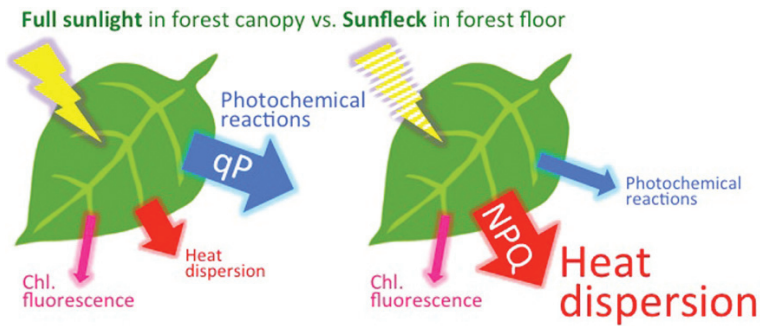
**Figure 6.** Relationships between photosynthetic rates and PPFD during measurements under different pulsed lighting cycles or continuous lighting of leaves grown under non-pulsed lighting conditions.  $\phi$  indicates the apparent quantum yield (the initial slope of response curve). The dotted vertical line shows the growth PPFD ( $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ).



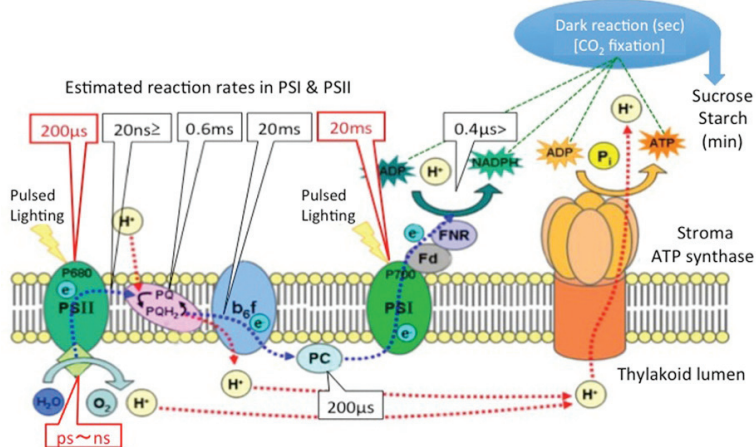
**Figure 7.** Chlorophyll fluorescent parameters of leaves grown under non-pulsed continuous lighting (horizontal line) and under different pulsed lighting cycles (○). Fv/Fm and Fv'/Fm' were measured with dark-adapted or exposing leaves to 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (saturated irradiance with or without pulse cycles) to estimate photochemical (qP) and non-photochemical (NPQ) quenching.

effects on the chlorophyll fluorescence parameters. Relative to continuous light, the pulse frequencies at 0.1, 1, 100 Hz and 1 kHz were reported to be optimal for growth, productivity and energy consumption [15]. However, earlier report suggested that tomato plants could use intermittent light (in kHz frequencies) as effectively as they use continuous light [16]. Flashing light has been useful as an experimental technique to extract additional information from photosynthesis measurements. The complex web of reactions in photosynthesis have different response times, so that fluxes through some reactions can be much faster than others resulting in fluctuating pool sizes. Furthermore, each reaction process seems to occur very rapidly in nanosecond to millisecond rates in the light reaction compared to seconds to minute rates in the dark reaction (**Figure 9**). When the light is delivered in pulse short enough to only allow a single turnover of PSII, the absorbed quanta are used with maximal efficiency until they





**Figure 8.** Photochemical reactions of photosynthesis of leaf lettuce are affected by exposing leaves to slow flash pulsed strong lighting (<1 Hz). An instantaneous response of PS by enhancing heat dissipation from leaves exposed to natural sunfleck in forest floor where plants are protected against photoinhibition.



**Figure 9.** Photochemical reactions of photosynthesis are connected by an electron transport chain (ETC) between Photosystem I (PSI) and II (PSII), and ATP synthase embedded in thylakoid membrane in chloroplast. ETC composes of PSII reaction center (P680), plastiquinone (PQ), cytochrome *b<sub>6</sub>f* complex (*b<sub>6</sub>f*), plastocyanin (PC), PSI reaction center (P700), ferredoxin (Fd), ferredoxin-NADP<sup>+</sup> reductase (FNR). Each estimated electron transfer rate is an example from Refs. [3, 4].

exceed the surface density of PSII [16]. Providing brighter flashes does not result in any additional photochemistry because the reaction centers do not reopen during the lifetime of the quanta in the pigment array. Consequently, it is possible to quantify the number of functional PSII reaction centers per unit leaf area in intact leaves [17]. With longer pulses of light, photochemical efficiency is reduced because a proportion of reaction centers are closed. However, photochemical efficiency expressed per open reaction center is still maximally efficient. Once a transthylakoid ΔpH gradient develops, photochemical efficiency of open centers begins to decline [18].

The photosynthetic responses to sudden changes in light conditions (lightflecks) were studied and the CO<sub>2</sub> uptake rate found to be maintained at a certain level for several seconds after a sudden decrease in light intensity [4, 1]. When photosynthetic rates have been measured with flashing light, considerable rate increases have been observed at high irradiances in the absence [19] or presence [20] of continuous background light. This has been attributed to post-illumination CO<sub>2</sub> fixation consuming the pools of RuBP [21] as well as triose phosphate that requires some extra ATP synthesis from the  $\Delta$ pH gradient to convert it to RuBP [22]. If the time between flashes is sufficiently long, the  $\Delta$ pH gradient is unable to build up as it is dissipated by the post-illumination CO<sub>2</sub> fixation's demand for ATP synthesis. This means that electron transport can occur more rapidly during the pulses of light than in continuous light because plastoquinone reoxidation is not restricted by the  $\Delta$ pH gradient. If a sufficiently large  $\Delta$ pH gradient exists at high irradiances even with high CO<sub>2</sub> partial pressures in leaf, then one would expect that flashing light could further enhance the photosynthetic rate. Stitt [23] observed a 30% increase in the rate at 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  with spinach when long flashing cycles (10s) were used in an oxygen electrode with 5% CO<sub>2</sub>, the leaf was kept for 10 s at each light condition. Roden and Percy [2] found that the efficiency of post-illumination CO<sub>2</sub> fixation only declined once the intervening dark period exceeded about 1 s. Kriedemann et al. [19] also showed that fluctuating light with 200 ms dark intervening periods enhanced photosynthesis. Electron transport during the flash reduces NADPH and builds up the pool of RuBP and triose phosphate. These pools, termed assimilatory power by Laisk et al. [21] enable post-illumination CO<sub>2</sub> fixation to occur and were equivalent to 5 s of photosynthesis in sunflower leaves at 100–200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD. Sharkey et al. [22] showed the rapid buildup and consumption of these pools during and after lightflecks. For *Phaseolus* leaves grown in sunlight, the RuBP pool was 5  $\mu\text{mol m}^{-2}$  and the total post-illumination CO<sub>2</sub> fixation was 12  $\mu\text{mol m}^{-2}$  when triose phosphate was included. The latter requires additional ATP synthesis that comes from the proton pool stored in the thylakoid lumen [22]. Steady-state pool sizes of 100  $\mu\text{mol m}^{-2}$  for RuBP have been measured in *Raphanus* leaves [24], which are certainly adequate to cope with the maximum of 5  $\mu\text{mol m}^{-2}$  observed here per flash. The balance between Rubisco activity and electron transport rate is effectively increased by the ratio of intervening time to flash length up to the limit set by the pool sizes of RuBP and triose phosphate. Therefore, in flashing light, the dependence of electron transport rate on CO<sub>2</sub> should be small. Thus, photosynthetic intermediates (PIs) were quickly produced by photochemical reactions during lightflecks and consumed thereafter in the CO<sub>2</sub> fixation occurred during next dark or dim light period. An actual estimation of PIs content is difficult under pulsed light, especially at high frequencies. A kinetic model to estimate Pn was developed by considering that photosynthetic intermediates were pooled during light periods and then consumed by partial photosynthetic reactions during dark periods [25]. According to this model, they quantitatively estimated the effects of pulsed light frequency and duty ratio on photosynthetic rates of cos lettuce leaves. The estimated Pn was lower, especially under pulsed light at lower frequencies and did not exceed Pn under continuous light. Accordingly, they concluded that, compared with a constant PPFD, fluctuation in PPFD can theoretically be disadvantageous to photosynthesis, even though the time-averaged PPFD are identical. In this study, lettuce leaves grown under pulsed light at low frequencies (2–0.5 Hz) maintained higher Pn compared to

leaves grown under continuous light when they were measured under pulsed light. It is suggested that photosynthetic adaptations to intermittent radiation might have occurred during leaf development, and that the adapted leaves can more efficiently use flashing light.

## 5. Conclusions

Pulsed light at high frequencies (2–20 kHz, 50% duty ratio,  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) positively affected the growth of lettuce leaves under controlled environment. The photosynthetic performances showed differences between leaves developed under pulsed light and leaves developed under continuous light, when the  $\text{CO}_2$  uptake rates and chlorophyll fluorescence parameters were measured at lower frequencies ( $<2 \text{ Hz}$ ). In the pulsed light technique, it is important to determine both optimal frequency and duty ratio for plants to attain the most efficient use of harvested light. The reason why growth was enhanced under pulsed light at high frequencies has not been resolved by analyzing photosynthetic performances in this study. Further research is required for detecting the pool size of PIs in leaves during their exposure to intermittent radiation. We propose that the pulsed lighting technique by using LEDs could become a useful for the production of leafy vegetables controlled plant factory systems in the near future.

## Abbreviations

photosynthetic photon flux density (PPFD)

photosystem I & II (PSI & PSII)

maximum quantum yield of PSII in dark-adapted state ( $F_v/F_m$ )

effective quantum yield of PSII induced in light ( $F_v'/F_m'$ )

non-photochemical quenching by heat dissipation (NPQ)

photochemical quenching as an estimate of open PSII reaction centers ( $q_P$ )

quantum efficiency of PSII ( $\Phi_{\text{PSII}}$ )

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