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Influence of Pulsed, Scanning and Constant (16- and 24-h) Modes of LED Irradiation on the Physiological, Biochemical and Morphometric Parameters of Lettuce Plants (*Lactuca sativa* L.) while Cultivated in Vertical Farms

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Abstract: In city farming, when growing green crops, a significant part of the production cost is the cost of electricity for lighting. The physiology, biochemistry, morphology and productivity of plants can be affected by changing irradiation modes and these changes reduce electricity costs. However, the results of studies in the literature are contradictory. In this work, we investigated the effect of impulse (frequency 1000 Hz and duty cycle 67%), scanning (the principle of running lights) and constant 16 h and 24 h modes of operation of white light LED irradiators on the physiological, biochemical and morphometric parameters of lettuce with red and green leaves. The daytime integral of light in all variants remained unchanged $\sim 15.6 \text{ mol m}^{-2} \text{ day}^{-1}$. Daily electricity consumption also did not differ significantly. Plants were grown on racks in a climatic chamber up to 35 days of age. For lettuce with red leaves, the most optimal for biomass accumulation and synthesis of anthocyanins was the impulse illumination mode, while for lettuce with green leaves, no statistically significant differences in biomass were observed under different irradiation modes. For red-leaved lettuce, it was found that the highest concentration of carotenoids in the leaf was observed under constant (24 h) and scanning irradiation, which is associated with a more active reaction of the photosynthetic system to prolonged irradiation and increased intensity during scanning irradiation. Also, increased photosynthetic activity was found in both varieties of lettuce at 16 h of operation of LED irradiators, which, however, did not affect their final productivity. The results may be useful for the development of LED illuminators for use in rack growing.

Keywords: pulsed LED light; continuous LED; scanning LED light; energy saving; lettuce; vertical farms; growth; cultivation

1. Introduction

During the cultivation of plants in vertical farms, the cost of electrical energy can be quite high due to the many hours of operation of the irradiation system, which can be a limiting factor for the development of technologies and the profitability of the farm. One of the ways to reduce the cost of electricity for plant irradiation is the use of radiation sources with an impulse mode, which saves up to 12% of electricity compared to the constant mode without reducing plant productivity by varying the duration of working cycles and the ratio of light and dark (l/d) periods in each cycle [1,2].

Energy savings are also achieved due to a slight increase in the light output of LEDs with impulse power. For example, Cree LEDs of the X-Lamp XP-C series, when powered by an impulse current with a frequency of 1 kHz with a duty ratio (D) of 5%, showed a

10–15% higher light output compared to continuous mode [3]. Also, impulse irradiation has an influence on plant physiology, biochemistry, morphology and productivity. Thus, in basil plants, impulse irradiation caused a significant increase in biomass by 47% compared to continuous light [4], while the overall rate of photosynthesis (P_n) did not significantly decrease [5]. Interesting results were obtained while wheat was irradiated with impulse light. Instead of a photoprotective reaction, an increase in photosynthetic carbon assimilation occurred [6]. The use of intermittent illumination with a frequency of 1 kHz and $D = 50\%$, 70% and 80% and with the same intensity on wheat plants showed that at $D = 80\%$ illumination, the plants showed the highest P_n , while the concentration of chlorophyll, antioxidant capacity, yield index and the weight of a thousand grains corresponded to the variant with constant illumination [7].

The etiochloroplasts defined from wheat leaves acquired green colour with repeated flashes of light at intervals of 15 min in the dark. These plastids exhibited light-induced decolourization of chlorophyll and carotenoids, and pigment stabilization occurred after additional repeated flashes with short intervals of darkness (5 s). However, when comparing plastids with similar photochemical activity, stabilization was much lower than under constant illumination [8]. Experiments carried out on pepper plants show that the addition of impulse radiation to the flux of fluorescent lamps affects the functional state of the photosynthetic apparatus—the efficiency of electron transport by open photosystem II (PSII) reaction centres and the quantum efficiency of PSII increased. Additional impulse irradiation contributed to an increase in the biomass and content of chlorophyll in the tissues of the pepper leaf blade [9].

A study of the response of tomato leaves to impulse irradiation showed that all light generated a photosynthetic photon flux (PPF) equivalent of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ from $5000 \mu\text{mol m}^{-2} \text{s}^{-1}$ pulses for $1.5 \mu\text{s}$ followed by periods of dark $148.5 \mu\text{s}$ ($D = 1\%$, frequency 6.6 kHz), did not affect photosynthesis compared to continuous illumination of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$. When $1/d$ pulses were extended to 2 ms of light and 198 ms of dark ($D = 1\%$, frequency 5 kHz), net photosynthesis decreased by a factor of two [10]. In this work, the theory was also confirmed that the pigments of the xanthophyll cycle were not affected by any of the impulse light modes.

There are quite a lot of works on the study of the effect of impulse light sources on the physiology, biochemistry, and morphology of various varieties and species of plants [11–16], but an all-round study of the effect on plant growth and their productivity has not been previously conducted. The parameters of irradiation modes that allow to reduce the consumption of electrical energy without loss in the quality of the grown products were not shown.

Another way to reduce energy costs during the artificial irradiation of plants is the use of scanning phytoemitters (Light-Mover). This is a device that combines a phyto-illuminator mounted on a rail attached to the ceiling of a greenhouse or growing room, along which the lamp is constantly moving electrically. The most popular and widespread are the scanning phytoirradiators moved linearly. There are also systems with a circular motion of the irradiators. To cover large areas, it is possible to use several parallel guides. The advantage of scanning phytoirradiators is a more uniform distribution of radiation over the growing area and a significant expansion of the irradiation zone of each phytoiradiator.

Manufacturers claim that such devices reduce lighting costs (for any type of lamp) or increase yields for equal energy costs. It has been established that shade-loving plants can quickly adapt to changing periods of diffuse and direct radiation under natural conditions [17]. Relatively few experiments have been carried out on photophilous plants that have shown the species-specific response of plants to scanning irradiation [18,19]. The results of studies on the use of impulse and scanning radiation systems in crop production are controversial and require further study. The effectiveness of such systems depends on many factors, such as plant species and variety, planting frequency, intensity and spectral composition of radiation. The purpose of this work is to study the time modes of LED irradiation (constant, impulse and scanning) of lettuce grown on racks.

The purpose of this work is to study the effect of impulse, scanning, and constant 16- and 24-h LED irradiation modes on the physiological, biochemical, and morphometric parameters of lettuce (*Lactuca sativa* L.) grown in vertical farms. In contrast to earlier studies, for the scanning mode of irradiation, we used LED irradiators, which have several advantages over gas discharge lamps. They can be placed closer to plants without causing leaf burns from excess radiation, have a longer lifespan, and are promising for vertical farms.

2. Materials and Methods

2.1. Characteristics of Lettuce Varieties

For our experiments, two varieties of lettuce were chosen—a semi-head green-leaved variety called ‘Azart’ and a red-leaved variety called ‘Lollo Rossa’. These varieties belong to different groups in terms of colour and structure, in addition, they are grown well in hydroponic culture and have been tested by us in previous experiments [20]. The ‘Azart’ lettuce variety (“Prestige” company)—mid-season semi-head lettuce. The rosette of leaves is semi-erect, 25–27 cm high, and 31–32 cm in diameter. The texture of the leaf tissue is tender and semi-crispy. The mass of a head of cabbage reaches 230 g ‘Lollo Rossa’ lettuce variety is a mid-season variety of leaf lettuce. The rosette of leaves is semi-erect, 20 cm high, 30 cm in diameter, weighing 200–350 g. Productivity is 3 kg/m².

2.2. Cultivation Conditions

The research was conducted in a climate chamber for growing green crops. Plants were grown in plastic trays on racks equipped with periodic flood hydroponic systems (Figure 1a). Seeds were sown in cubes with a mineral wool substrate. To avoid the negative effect of far-red radiation on seed germination, plant lighting was turned off before germination in the climatic room. After the appearance of the cotyledons, the lighting was turned on again. After seedlings were found and the first true leaves appeared, three plants were left in each cube. For growing plants, seven light-insulated racks of three tiers each were used. The cultivation area for each light treatment was 0.68 m². The planting area was 50 plants per 1 m². The microclimate in the room was maintained by an automatic system. The day/night air temperature was 25/22 °C with a relative humidity of 75%. The ventilation system maintained the concentration of carbon dioxide and corresponded to atmospheric values of 400–450 ppm. Additional CO₂ was not used. Nutrient solutions were prepared using Flora Series[®] hydroponic fertilizer complex (Terra Aquatica, Fleurance, France). The electrical conductivity of the nutrient solution was maintained within 1500–1600 µS/cm.

2.3. Irradiation Conditions

Irradiation of plants was carried out by irradiators based on white light LEDs with a colour temperature of 4000 K Samsung LM281b+ 2835 (Seoul, South Korea). The specific power consumption per one shelf of the rack 0.68 m² was 90 W m⁻². The daytime integral of light (DLI) in all variants of the experiments was ~15.6 mol m⁻² day⁻¹. Measurements of the photon flux density and the spectral composition of irradiation were carried out using an MK350D Compact Spectrometer (UPRtek Corp. Miaoli County, Taiwan). The spectral composition of the radiation is presented in Figure 1b.

On the first rack, round-the-clock irradiation of plants was implemented 24 h a day. This mode was chosen because some studies on growing plants with a long photoperiod show some potential advantages of a 24-h irradiation regimen [21,22]. The PAR irradiance was ~180 µmol s⁻¹ m⁻². Daily electricity consumption was 5.7 kWh.

On the second rack, impulse irradiation of plants with a frequency of 1 kHz and D = 67% was implemented since early studies showed the maximum efficiency of light use in this mode of lettuce illumination [23]. The PAR irradiance (average value) was ~180 µmol s⁻¹ m⁻². Daily electricity consumption was 6.1 kWh.

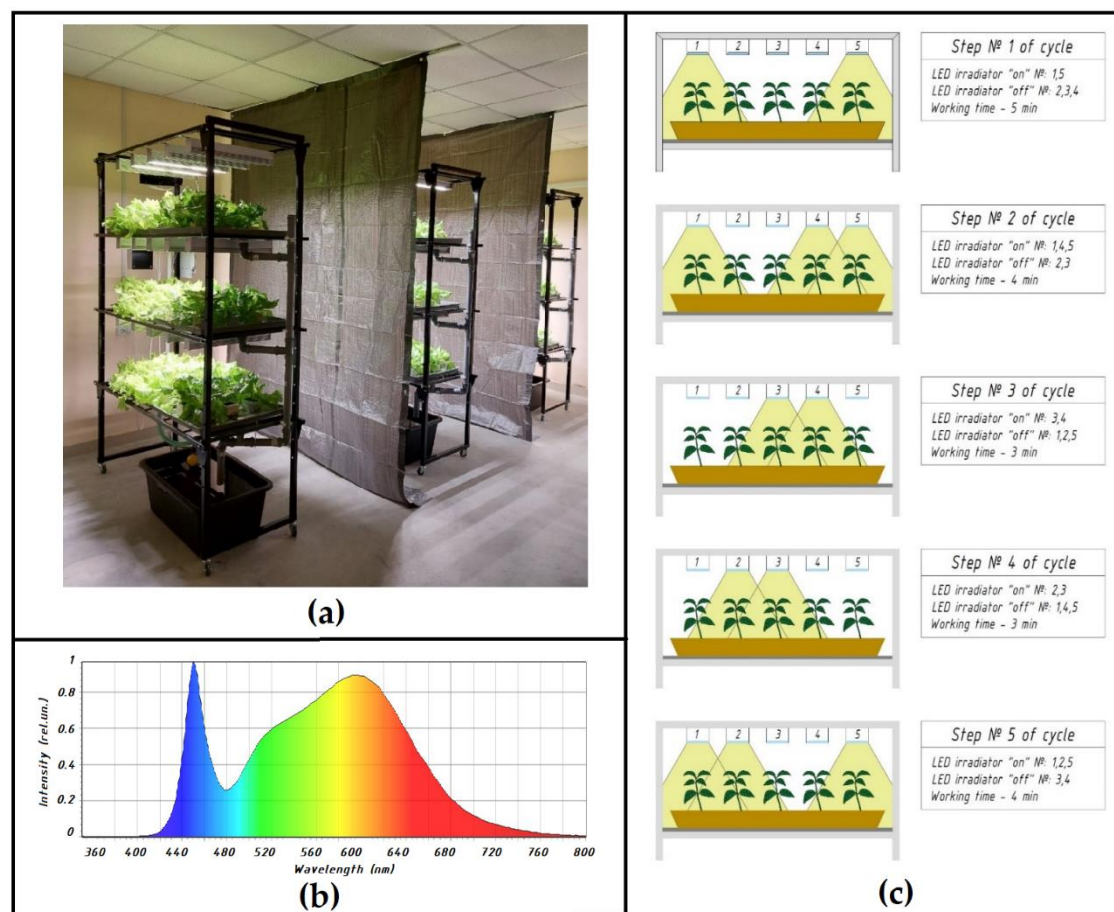


Figure 1. Climatic chamber with plants (a), the spectral composition of phytoirradiators radiation (b), operating parameters of scanning irradiation (c).

On the third rack, scanning irradiation of plants was implemented with five irradiators, which were switched on in pairs according to the “running lights” principle. The work cycle was divided into five steps (Figure 1c). The duration of each step was adjusted so that DLI for each plant on the shelf was $\sim 15.6 \text{ mol m}^{-2} \text{ day}^{-1}$. The PAR irradiation at the plant level varied from $\sim 45 \mu\text{mol s}^{-1} \text{ m}^{-2}$ to $\sim 285 \mu\text{mol s}^{-1} \text{ m}^{-2}$. Daily electricity consumption was 5.8 kWh.

The lettuce of ‘Azart’ and ‘Lollo Rossa’ varieties were irradiated 16 h a day on the fourth rack (control variant). The PAR irradiance was $\sim 275 \mu\text{mol s}^{-1} \text{ m}^{-2}$. Daily electricity consumption was 6.0 kWh.

2.4. Biometric Measurements of Lettuce Plants

On the 25th and 35th days of cultivation, the fresh and dry mass of the aerial parts of plants was weighed using a Sartorius LA230S balance (Laboratory Scale, Göttingen, Germany). To obtain dry matter, the selected plants were dried to constant weight in an oven at a temperature of 105°C . The leaf area was determined on an LI-COR LI-3100 AREA METER photoplanimeter (LI-COR, Inc., Lincoln, NE, USA). The nitrate concentration was measured by an ionometric method using the ion-meter “Itan” (Tom’analit, Tomsk, Russia).

2.5. Spectrophotometric Measurements of the Pigment Composition of Leaves

Quantitative pigments were analysed by extracting them from plant tissues with solvents. The optical density of the pigment extract was determined on a SPECS SSP-705 (Moscow, Russia) spectrophotometer at wavelengths 662, 644, and 440 nm using cuvettes with an absorbing layer thickness of 10 mm. The concentration of chlorophylls a, b and

carotenoids was calculated using the Holm–Wettstein formula for 100% acetone [24]. The measurements were carried out on the 25th and 35th days of cultivation.

2.6. Reflection Measurements in Lettuce Leaves

Leaf reflectance spectra were measured using a portable PolyPen RP 410 UVIS system (Photon Systems Instruments, Drásov, Czech Republic). Leaf reflectance measurements were taken 35 days after the start of cultivation. The spectra of ten plants were measured under each illumination and at each cultivation period. Three spectral measurements were carried out on different leaves of each plant.

Using the PolyPen RP 410 UVIS software, the main reflectance indices were automatically calculated. In this case, we analysed, first of all, the normalized difference vegetation index (NDVI) [25], which is a widely used indicator of the photosynthetic biomass of plants, the Zarco-Tejada–Miller index (ZMI) [26] and Gitelson–Merzlyak Indices 1 and 2 (GM1 and GM2) [27], which are highly sensitive to the content of chlorophylls, carotenoid reflectance indices 1 and 2 (CRI1 and CRI2) [28], which are sensitive to the content of carotenoids, and anthocyanin reflectance index (ARI) [29], which is sensitive to the content of anthocyanins.

2.7. Measurement of Chlorophyll Fluorescence Parameters

To measure the activity of the light stage of photosynthesis, a portable fluorimeter FluorPen FP 110/S (Photon Systems Instruments, Drásov, Czech Republic) was used to detect active chlorophyll fluorescence and its further analysis using the PAM method or the OJIP test. FluorPen FP 110/S includes a detector (PIN photodiode with a narrow band filter, working optical range from 667 to 750 nm) and a blue LED emitter (maximum about 455 nm), an ambient light sensor. To assess F_v/F_m , the leaf was preliminarily dark-adapted for at least 20 min [30].

2.8. Raman Spectroscopy of Lettuce Leaves

Analysis of the Raman spectra (RS) was carried out on the 25th day of cultivation, on undisturbed samples of living tissues of lettuce plants. The measurements were carried out on a Senterra II confocal Raman microscope (Bruker, San Jose, CA, USA) using a red (785 nm) laser. Additional processing of the samples was not carried out, the accumulation time and the number of additions did not change, and corrective substrates (SERS) were not used in the measurements. The resolution was 4 cm^{-1} . The measurements were carried out in quadruple repetition.

2.9. Statistical Data Processing

All experiments were carried out threefold. Statistical processing of measurement results and plotting were performed in Python 3.9 and MS Excel. To estimate the statistical significance by the considered parameters the Independent two-sample *T*-test at $p < 0.05$ significant levels.

3. Results and Discussion

3.1. Morphological Parameters of Lettuce Plants

According to biometric indicators, the studied lettuce varieties had different responses to irradiation regimes (Table 1). The indicator of the accumulation of a fresh mass of plants for the semi-head ‘Azart’ variety had the highest value with continuous illumination for 24 h, and for the ‘Lollo Rossa’ leaf variety—when using impulse irradiation; at the same time, the differences between these lighting options remained unreliable. The obtained results are consistent with similar studies carried out on red leaf lettuce (*Lactuca sativa* L. cv. ‘Sunmang’), the biomass of which did not differ significantly from the control with continuous illumination at a duty cycle of 1 kHz ($D = 75\%$) [2]. In general, the fresh mass of both lettuce cultivars was somewhat less under scanning irradiation, as was the case in studies conducted on rose and bluebells plants [18,19].

Table 1. Morphological parameters of lettuce plant of ‘Azart’ and ‘Lollo Rossa’ varieties on the 35th day of cultivation. Values represent mean SEM (n = 10). Letters indicate significant differences among treatment and control samples ($p < 0.05$).

Irradiation Mode	Fresh Mass, g	Dry Mass, %	Leaf Surface Area, cm^2	Stem Height, cm
‘Azart’				
Constant (24 h)	42.17 (a) ± 11.46	6.31 (a) ± 1.05	954.74 (a) ± 188.3	3.65 (ab) ± 1.28
Impulse	36.22 (a) ± 9.17	6.56 (a) ± 0.65	816.66 (a) ± 174.68	2.75 (b) ± 0.69
Scanning	35.06 (a) ± 7.07	4.87 (b) ± 0.47	869.36 (a) ± 146.38	5.69 (a) ± 1.62
Constant (16/8 h) (control)	36.77 (a) ± 7.61	5.12 (ab) ± 0.62	893.38 (a) ± 165.66	3.19 (b) ± 0.85
‘Lollo Rossa’				
Constant (24 h)	31.42 (ab) ± 7.22	6.48 (a) ± 0.55	937.51 (ab) ± 163.05	2.8 (a) ± 0.54
Impulse	40.39 (a) ± 10.2	6.53 (a) ± 0.56	1073.66 (a) ± 241.45	2.47 (a) ± 0.68
Scanning	25.76 (b) ± 5.33	5.9 (b) ± 0.33	747.13 (b) ± 145.44	1.53 (b) ± 0.29
Constant (16/8 h) (control)	32.6 (ab) ± 5.84	6.04 (ab) ± 0.55	945.78 (ab) ± 158.25	1.36 (b) ± 0.44

According to the accumulation of dry mass, both varieties showed a similar reaction depending on the lighting regime. In the control variant (16/8) and the variant with the use of scanning irradiation, the ratio of dry to fresh mass did not have statistically significant differences, however, in the variant with continuous illumination for 24 h and using the impulse irradiation mode, the accumulation of dry matter of the ‘Azart’ variety was significantly increased by 23% and 28%, respectively, and for ‘Lollo Rossa’ the increase in dry weight was not statistically significant. The studied lighting regimes had a different effect on the development of the leaf apparatus of plants of the ‘Lollo Rossa’ variety and did not have a significant effect on changes in the area of the leaf surface of the ‘Azart’ variety. The greatest increase in the leaf surface area of the ‘Lollo Rossa’ variety (13.5%) was observed in the variant with impulse irradiation, while scanning irradiation significantly reduced this indicator compared to the control variant of illumination (by 21%). The head lettuce variety ‘Azart’ in the variant with the scanning mode of lighting was significantly extended, while the height of the stem increased by 1.8 times and the rosettes of the leaves were non-compact, the plants acquired an unmarketable appearance. Studies of scanning irradiation modes on flower crops showed a different effect: when growing a rose, the height of the stem remained unchanged [18], and when growing a bell, it even significantly decreased [19]. This difference is due to the fact that for these flower crops, scanning irradiation was used as an addition to the main one, and in our case, it was the main one. A significant elongation of the stem of the ‘Lollo Rossa’ lettuce variety was observed in the variants with impulse and constant 24-h irradiation by 1.8 and 2 times, respectively.

3.2. Biochemical Parameters of Lettuce Plants

In the phase of active growth on the 25th day of cultivation, for ‘Lollo Rossa’ leaf lettuce in terms of concentration and ratio of pigments, the options with constant illumination 16/8 and impulse were optimal. The plants grown under these light modes had the highest content of total chlorophyll—1.2 mg per 1 g of fresh mass (Figure 2a). The ratio of total chlorophyll to carotenoids was more than four (Figure S1b) which corresponds to the period of active vegetation. As for the semi-head ‘Azart’ variety, there were no

significant differences in the ratio of chlorophylls to carotenoids (Figure S1a,b), and the highest concentration of pigments was observed in the control variant.

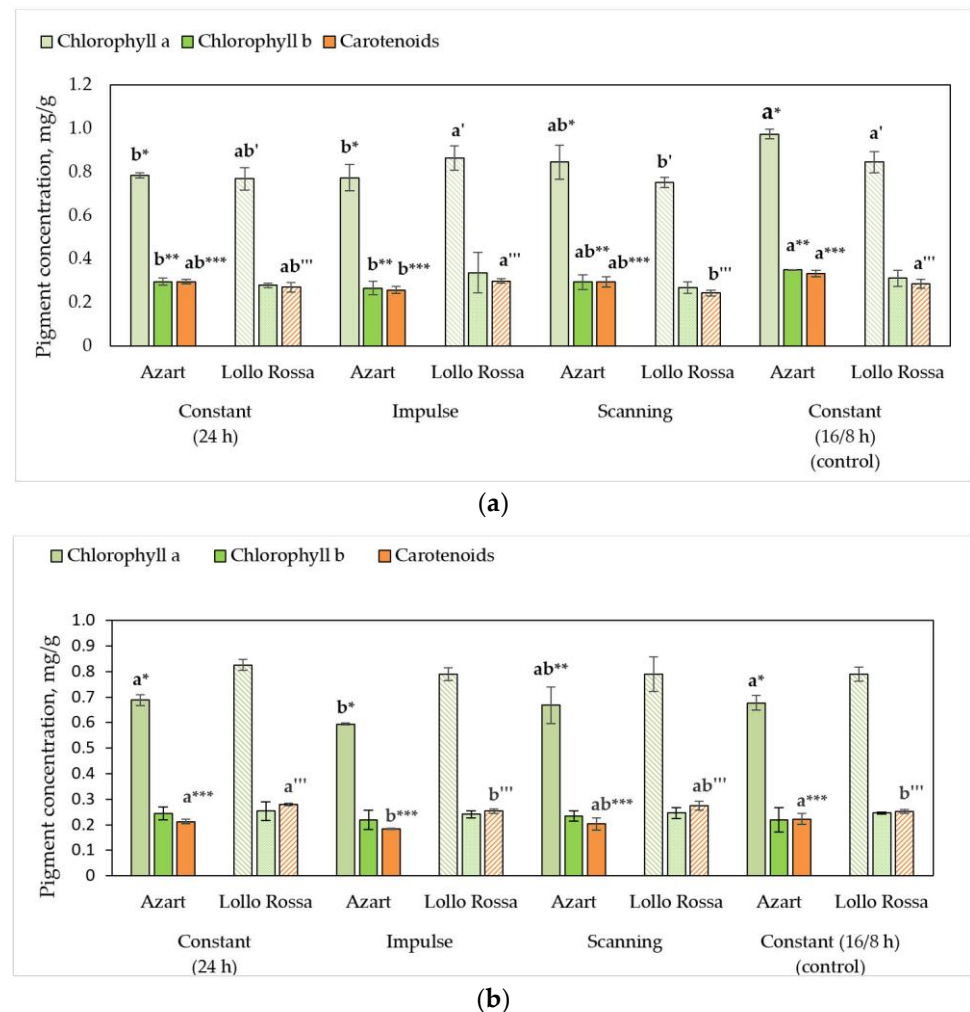


Figure 2. The concentration of pigments on the 25th (a) and 35th (b) day of cultivation. The solid colour indicates the columns of the diagram related to the 'Azart' variety; the shading columns of the diagram indicate data related to the 'Lollo Rossa' variety. Values represent mean SEM (n = 10). Letters indicate significant differences among treatment and control samples ($p < 0.05$). Asterisk sign near letters is used for the 'Azart' variety and apostrophe - for the 'Lollo Rossa' variety. One sign is used for Chlorophyll a, two signs – for Chlorophyll b and three signs – for Carotenoids.

On the 35th day of cultivation, the 'Lollo Rossa' leaf lettuce was distinguished by a more intense accumulation of pigments (Figure 2b). This may be due to the screening effect of anthocyanins [31]. It also accumulated more carotenoids in relation to the total content of chlorophylls in the variants with Constant 24 and Scanning irradiation (Figure S1d), what indicates increased stress level since carotenoids protect the photosynthetic apparatus from negative light exposure [32]. Plants of the 'Azart' variety in the variant with the use of impulse irradiation were distinguished by a reduced concentration of photosynthetic pigments. The ratio of chlorophyll a to chlorophyll b (Figure S1c) in the half-head 'Azart' variety in all variants, except for the Constant 24, was below three, which indicates a slight lack of illumination, and the total chlorophyll content ratio to the carotenoid content was the highest under Impulse and Scanning irradiation modes. Thus, judging by the pigment composition, both constant 16/8 and impulse irradiation modes are suitable for 'Lollo Rossa' leaf lettuce and Constant 24 for the 'Azart' variety.

It is known that the prolongation of the photoperiod can increase the fresh weight and chlorophyll content, meanwhile, a decrease in the photoperiod helps to reduce the content of nitrates in lettuce [33]. In this regard, we checked the content of nitrates in plants at the time of green mass harvesting. The concentration of nitrates was minimal in the control variant and the variant with the use of impulse irradiation (Figure 3). When using the scanning and constant 24 modes, the concentration of nitrates was slightly higher but did not exceed the maximum allowable concentrations for green products. For these lighting options, the absence of a night period contributes to a greater accumulation of nitrates in the leaves of crops. The observed results are a consequence of the influence of the light environment on plant metabolism and carbon and nitrogen exchange processes. The data presented in this article correlates with previous studies [33,34].

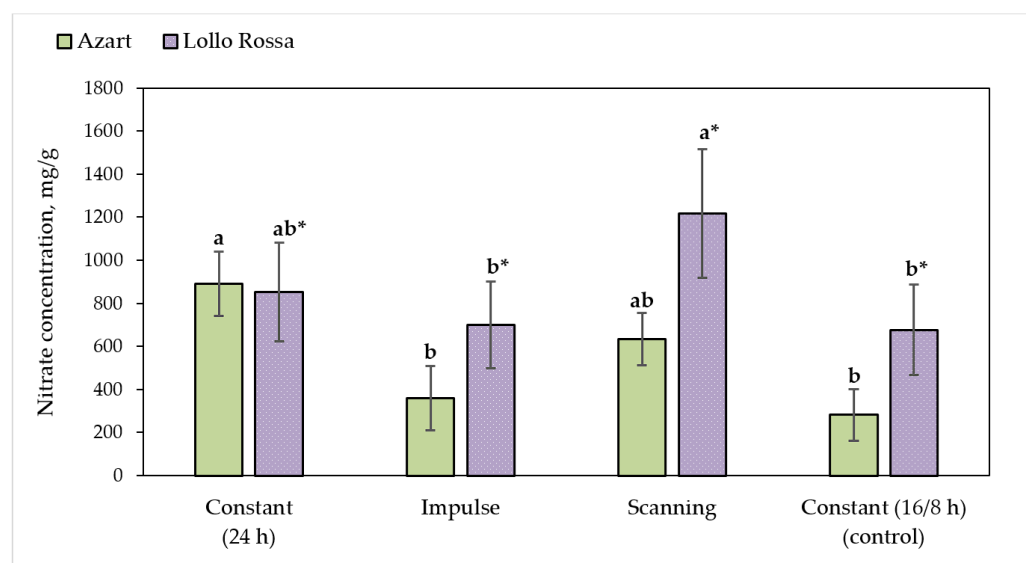


Figure 3. The concentration of nitrates on the 35th day of cultivation. Values represent mean SEM (n = 5). Letters indicate significant differences among treatment and control samples of ‘Azart’ variety, letters with asterisk sign—of ‘Lollo Rossa’ variety ($p < 0.05$).

3.3. Chlorophyll Fluorescence Parameters

At the next stage of the study, the influence of the illumination mode on the magnitude of the photosynthetic electron flux through photosystem II (ETR) and non-photochemical fluorescence quenching (NPQ) was studied.

It was shown (Figure 4a) that the lighting regime did not cause significant changes in the ‘Azart’ lettuce variety; at the same time, there was a trend towards an increase in ETR with increasing actinic light intensity in plants cultivated under continuous light conditions (control). In the ‘Lollo Rossa’ variety, this increase in the ETR of plants grown under continuous illumination was significant (Figure 4b). NPQ values at intermediate light intensities were reduced in both ‘Azart’ leaves (Figure 4c) and ‘Lollo Rossa’ leaves (Figure 4d); under conditions of low or high intensity of actinic light, the effect was absent. At the same time, there were practically no differences between the studied photosynthetic parameters in plants grown under other lighting conditions that were used in the work. These results are consistent with data obtained from red leaf lettuce, where there was no significant difference in the total rate of leaf photosynthesis under impulse illumination with $K_f = 75\%$ compared to continuous illumination with LEDs [2], and on green lettuce, where impulse irradiation did not significantly affect the concentration of chlorophyll and fluorescence parameters, except for the lowest frequency (0.2 Hz) [16].

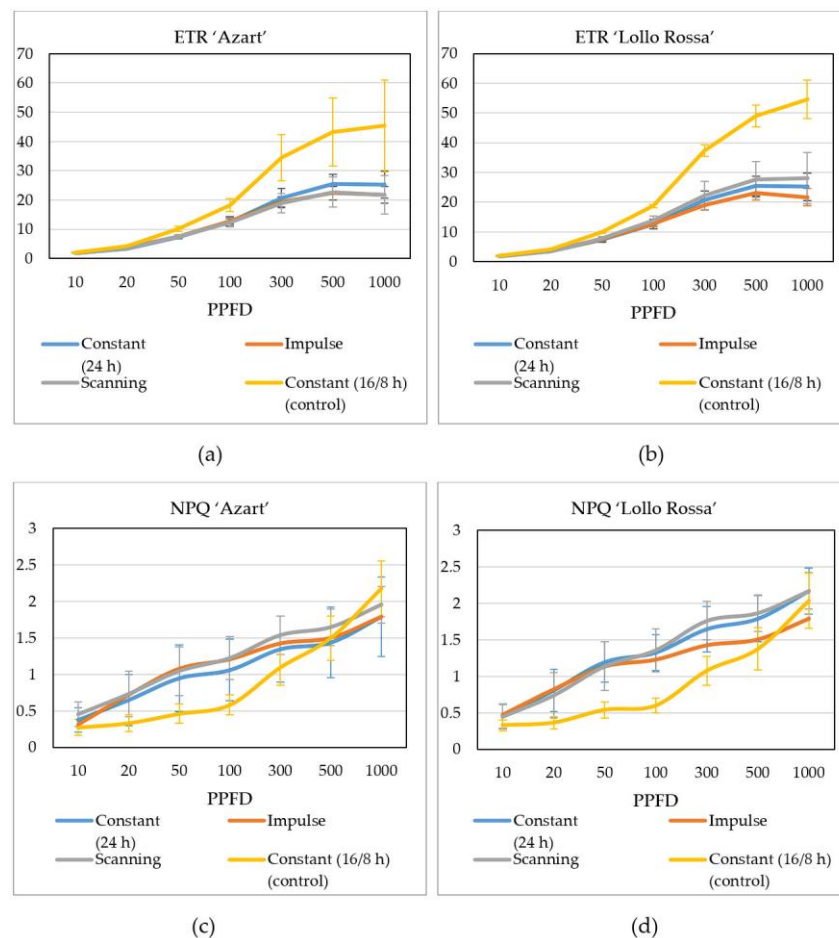


Figure 4. Dependence of the rate of electron transport (a,b) and non-photochemical (c,d) quenching of chlorophyll fluorescence on PPFD of lettuce plants on the 35th day of cultivation. Values represent mean SEM (n = 6).

The obtained results suggest that it is the high ETR value and low NPQ that can be the reason for the high productivity of lettuce plants under continuous illumination (Table 1); however, they do not explain the high efficiency of impulse lighting, since the photosynthetic parameters when grown under such conditions do not differ from the options with scanning lighting or simulated daylight hours (l/d = 16/8 h).

3.4. Study of the Influence of the Light Regime during Cultivation on Leaf Reflectance Indices

Analysis of the studied reflectance indices (NDVI, ZMI, GM1, GM2, CRI1 and CRI2) (Figure S2a,b) showed that their values were somewhat lower in the leaves of the 'Lollo Rossa' lettuce variety compared to the leaves of the 'Azart' variety. This result was in good agreement with the lower values of the chlorophyll index and the net productivity of photosynthesis in the 'Lollo Rossa' variety (Table 1 and Figure 2) since the studied indices correlate with the content of photosynthetic pigments.

Comparison of NDVI, ZMI, GM1, GM2, CRI1 and CRI2 indices in lettuce leaves of the same variety grown under different lighting conditions did not show statistically significant differences between the studied groups. At the same time, with the use of impulse and scanning irradiation, the 'Azart' variety showed a tendency to decrease in several indices sensitive to the concentration of chlorophyll in plants (NDVI, ZMI, GM1 and GM2), which is consistent with a decrease in the content of chlorophyll (Figure 2). Similar trends were also observed for carotenoid-sensitive indices CRI1 and CRI2 (Figure S2b), which correlate with changes in carotenoid concentration (Figure 2).

Even though the changes in the studied reflectance indices were not statistically significant, the observed trends corresponded well to changes in the content of photosynthetic pigments under different lighting conditions: a decrease in the content of chlorophyll *a* was accompanied by a decrease in the reflectance indices NDVI [25], ZMI [26], GM1 and GM2 [27], while a decrease in the content of carotenoids led to a decrease in CRI1 and CRI2 [28]. At the same time, small values and low significance of changes showed a relatively weak effect of the lighting regime on the reflectance spectrum of the sheet; Given the more pronounced differences in indices between the studied lettuce varieties (Figure S2a,b), the variety factor seems to be more significant for reflectance indices than the light regime during cultivation. On the other hand, Table 1 and Figure 2 show that under different growing regimes, differences in morphometric parameters of plants were observed, which suggests that such a difference may be associated with a change in the anatomical parameters of the leaf. Such features can significantly depend on the illumination parameters [35,36] and modify the reflectivity of the sheet [37] by decreasing or increasing changes in the reflectance indices.

For the “Lollo Rossa” lettuce variety, which has a high content of anthocyanins, the anthocyanin content index (ARI) was additionally measured (Figure 5). It was shown that the impulse lighting mode led to the increase in this index and has a positive effect on the anthocyanin concentration in lettuce leaves.

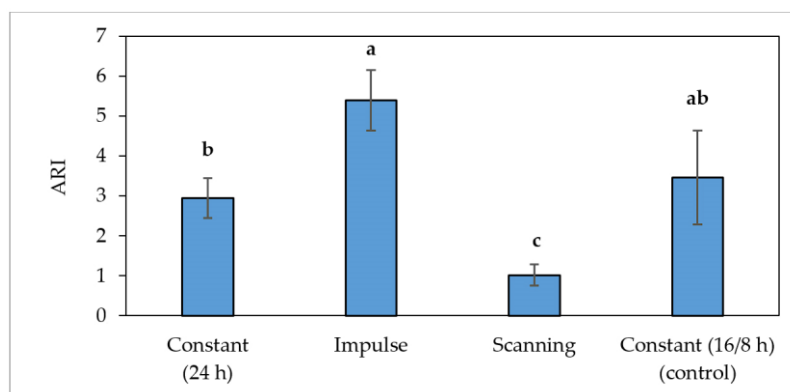


Figure 5. Anthocyanin content index (ARI) of lettuce plants on the 35th day of cultivation. Values represent mean SEM ($n = 10$). Letters indicate significant differences among treatment and control samples ($p < 0.05$).

3.5. Raman Spectroscopy of Lettuce Leaves

Usually, as a plant culture in Raman spectroscopy, *Lactuca sativa* is used to determine toxicants, plastics and heavy metals [38–40]. To identify the optimum points for collecting the Raman spectrum for both varieties, spectral data were collected from the inner space of the cell, the centre of the open stomata, and its valve. Based on the data obtained, the most informative were the spectra of the intracellular space (Figure 6).

Most often, peaks associated with pigments, especially with carotenoids and chlorophylls, can be detected in the spectra of plant samples. The main pigment peaks are observed in the range of 1500–1550 and 1150–1170 cm^{-1} , this part of the range is associated with in-phase stretching oscillations C=C and C-C of the polyene chain. In addition, swing patterns in the plane of CH_3 groups attached to the polyene chain can be identified as peaks of average intensity in the range of 1000–1020 cm^{-1} [41]. Assessing the average spectra of ‘Azart’ and ‘Lola Rossa’ lettuce varieties, it should be noted that the overall colour and content of pigments significantly affected the data: the leaves of plants of the ‘Lola Rossa’ variety showed a higher and noisier spectrum, with isolated peaks characteristic of photosynthetic pigments and organic acids (Figure S3). In general, the intensity of the red-leaf ‘Lola Rossa’ lettuce variety detected by CR was more than two times higher than that of the green-leaf ‘Azart’ variety, which is associated with an increased content

of carotenoids and anthocyanins (Figure 2), which have the properties to raise the overall intensity of the spectrum. A change in the illumination mode had little effect on the cell biochemistry reflected in the spectral data. This was more pronounced in the leaves of 'Lola Rossa' variety lettuce, which is explained by a change in the concentration of anthocyanins with a change in daylight hours (Figure 7).

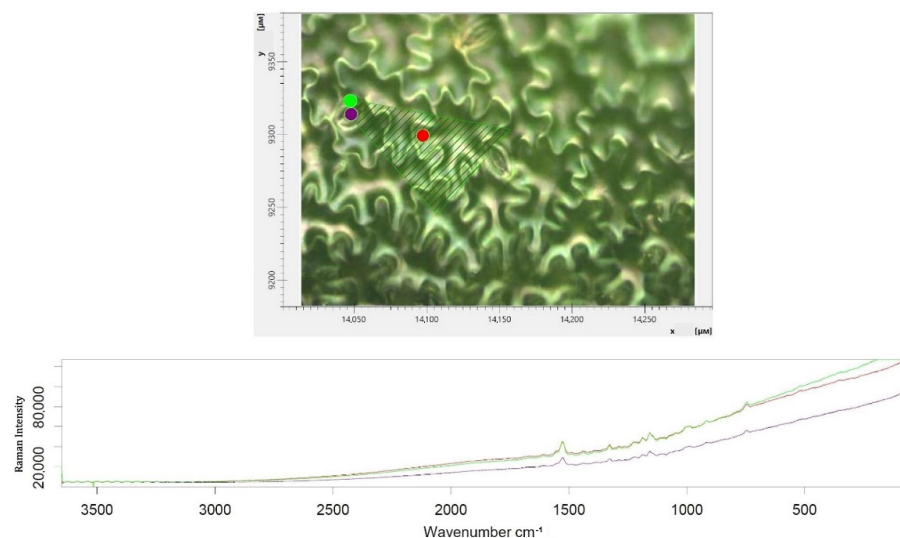


Figure 6. Measurement and selection of points on a lettuce leaf of the 'Azart' variety on the 35th day of cultivation, where red is the intracellular space, purple is the centre of the stomata, and green is the stomatal valve.

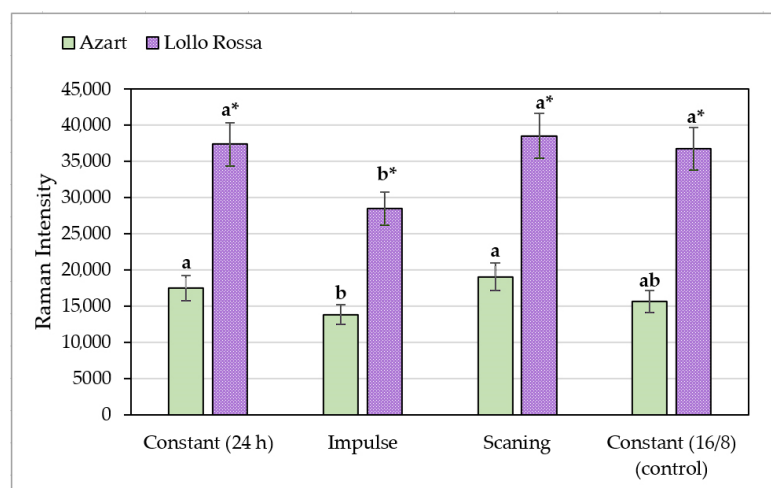


Figure 7. Varieties and variations in average intensity of significant peaks of the 'Azart' variety and 'Lola Rossa' on the 35th day of cultivation. Values represent mean SEM ($n = 5$). Letters indicate significant differences among treatment and control samples of 'Azart' variety, letters with asterisk sign—of 'Lollo Rossa' variety ($p < 0.05$).

As a result of the analysis of various types of lighting, it was found that the most pronounced spectra are plants grown under the scanning and constant type of lighting, which indicates a greater overall biochemical activity, expressed in an increase in the concentrations of pigments, sugars and organic acids in plant cells. The spectral data for both varieties of lettuce correlate with each other (Figure 7).

4. Conclusions

For the semi-head ‘Azart’ lettuce variety, the largest accumulation of the fresh mass of the plant was recorded on the stand with constant irradiation (24 h), and the smallest under scanning illumination. For the red-leaved ‘Lollo Rossa’ lettuce variety, the accumulation of fresh mass under constant (24 h), impulse and constant (16/8 h) were approximately the same values with a slight advantage in impulse irradiation, and the smallest value was also recorded for the scanning variant. From the point of view of qualitative analysis, the highest concentrations of carotenoids in the ‘Lollo Rossa’ lettuce variety were found for constant (24 h) and scanning irradiation, since carotenoids protect the photosynthetic apparatus from negative and intense light exposure. The highest value of chlorophyll content was found for plant variants under constant (16/8 h) and impulse irradiation. It was shown that impulse lighting during plant cultivation led to a statistically significant increase in the anthocyanin content index (ARI), which indicates a positive effect of impulse lighting on the concentration of anthocyanins in lettuce leaves. For the ‘Azart’ lettuce variety, the minimum value of the pigment concentration was found for impulse irradiation, for constant (24 h), scanning and constant (16/8) irradiation, the obtained values did not differ significantly. Thus, judging by the accumulation of fresh mass and the pigment composition, constant (16/8 h) and impulse irradiation is suitable for the ‘Lollo Rossa’ lettuce variety.

For the semi-head ‘Azart’ variety, the options with constant (24 h) and impulse irradiation are optimal. Further research will be aimed at researching and evaluating the modes and methods for introducing impulse irradiation for growing leafy crops in greenhouses.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture12121988/s1>, Figure S1: The ratio of the concentrations of chlorophyll a to chlorophyll b on the 25th day (a) and the 35th day (c) of ‘Azart’ and ‘Lollo Rossa’ lettuce variety cultivation; the ratio of the concentrations of total chlorophyll to carotenoids on the 25th day (b) and the 35th day of cultivation (d) using different LED irradiation time modes; Figure S2: Vegetation indices of lettuce leaves NDVI, ZMI, GM1, GM2 (a) and CRI1, CRI2 (b) of ‘Azart’ and ‘Lollo Rossa’ varieties on the 35th day of cultivation using different LED irradiation time modes. Values represent mean SEM (n = 10); Figure S3: Comparison of normalized Raman spectra of ‘Azart’ (a) and ‘Lola Rossa’ (b).

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