

Article

Yield and Quality of Romaine Lettuce at Different Daily Light Integral in an Indoor Controlled Environment

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Abstract: In this study, the effect of different photosynthetic photon flux density (PPFD) provided by LEDs (Light Emitting Diodes) and photoperiod on biomass production, morphological traits, photosynthetic performance, sensory attributes, and image texture parameters of indoor cultivated romaine lettuce was evaluated. Two cultivars of lettuce *Lactuca sativa* var. *longifolium* namely ‘Casual’ (Syngenta)—midi romaine lettuce with medium-compact heads—and ‘Elizium’ (Enza Zaden)—a mini type (Little Gem) with compact heavy heads—were used. PPFD of 160 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and photoperiod of 16 and 20 h were applied, and Daily Light Integral (DLI) values were 9.2, 11.5, 13.8, and 17.3 $\text{mol m}^{-2} \text{day}^{-1}$. The experiment lasted 30 days in the Indoor Controlled Environment Agriculture facility. DLI equal to 17.3 mol m^{-2} per day for cv. ‘Casual’ and 11.5–17.3 mol m^{-2} per day for cv. ‘Elizium’ allowed to obtain a very high fresh weight, 350 and 240 g, respectively, within 30 days of cultivation in an indoor plant production facility. The application of the lowest PPFD 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 16 h photoperiod (9.2 mol m^{-2} per day DLI) resulted in the lowest fresh weight, the number of leaves and head circumference. The level of nitrate, even at the lowest DLI, was below the limit imposed by European Community Regulation. The cv. ‘Elizium’ lettuce grown at PPFD 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 16 h photoperiod had the highest overall sensory quality. The cv. ‘Casual’ lettuce grown at PPFD 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 20 h photoperiod had the lowest sensory quality. The samples subjected to different photoperiod and PPFD were also successively distinguished in an objective and non-destructive way using image features and machine learning algorithms. The average accuracy for the leaf samples of cv. ‘Casual’ lettuce reached 98.75% and for cv. ‘Elizium’ cultivar—86.25%. The obtained relationship between DLI and yield, as well as the quality of romaine lettuce, can be used in practice to improve romaine lettuce production in an Indoor Controlled Environment.

Keywords: artificial lighting; quality features; image processing; sensory analysis; indoor farming; *Lactuca sativa* var. *longifolia*



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1. Introduction

An indoor closed plant cultivation system has recently been of interest to societies due to the production of high-quality plants with minimal use of resources and emissions of environmental pollutants [1]. The advantage of this system is the possibility of shortening of culture period by 40–50% with uniform growth and high-quality produce through the year. The light environment is one of the most important factors, which regulates biomass production, morphological traits of plants, and food quality [2–6]. High initial investment and operating costs of plant production in an indoor farming system require the maximization of yields obtained in a short time. Thus, optimizing light conditions is

imperative for year-round and efficient indoor crop production [4,7,8]. Lamps are the sole source of illumination in an indoor plant production system. Among artificial light sources, LEDs present the maximum high photosynthetically active radiations (PAR) efficiency and allow to optimize irradiance level for biomass production and energy costs [1,3,4].

Lettuce is one of the main crops in the plant factory. Several studies have investigated the impact of the photosynthetic photon flux density (PPFD) and photoperiod, as well as the interaction of these factors, on the growth and attributes of lettuce leaves produced in an indoor cultivation system [5,9–11]. Many scientific reports carried out on lettuce (*Lactuca sativa* L.) as a model plant grown in facilities without sunlight have shown that changes in the light spectrum significantly affect the growth and development of plants [10,12], their mineral composition [7,13,14], and nutrient content [2,15]. However, little information is available on the influence of treatment of light factors and photoperiod of romaine lettuce on the sensory quality. The sensory quality of food products can be determined using the attributes related to color, texture, smell, and taste [16]. The results found in the literature were related to the effect of the combined RBW (red (R), blue (B), and white (W)) LEDs on some sensory attributes of Boston lettuce [12]. Few studies relating to light requirements in an indoor plant production system have concerned head-forming as romaine lettuce *Lactuca sativa* var. *longifolia* [7,17–20]. It has been demonstrated that with an increase in the PPFD in the range from 100 to 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 14 h photoperiod, the biomass of above-ground part of loose-headed romaine lettuce ‘Lvling’ increased, and nitrate decreased [21]. The nitrate concentration in lettuce grown in hydroponics can approach a level considered hazardous for human health; therefore, it is essential for food safety to limit nitrate accumulation in leaves. A photoperiod of 14 h at 25/18 °C was the most suitable for hastened growth of romaine lettuce in a home hydroponic cultivation system [22]. In romaine lettuce, e.g., grown at 195 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, the morphological alteration and leaf necrotic spots, as well as tipburn, can be observed, which negatively affected the marketability of the produce [7]. The tipburn problem is genotype-dependent [23] and affects mainly head-forming lettuces, such as crisphead lettuce and romaine lettuce [24]. Xu et al. [19] showed that tipburn occurred after about three weeks of cultivation of romaine lettuce and its occurrence was positively correlated with light intensity and relative growth rate (RGR). Thus, by applying different light intensities and/or photoperiods, plant growth could be regulated, thus the tipburn occurrence and plant quality could be controlled.

Daily light integral, DLI (the product of photosynthetic photon flux density, PPFD and photoperiod), is used to determine the optimal quantity of light reaching plants. DLIs of 12–17 mol m^{-2} per day are generally recommended for green-leaved lettuce in vertical farming in terms of energy-saving, however, these DLI values vary among species and cultivars. For red-leaved lettuce type Batavia ‘Blackhawk’, DLI of 22.4 mol m^{-2} per day gave the best results in terms of improving photosynthetic traits and enhancing both production and qualitative traits [25], and a minimum DLI of 6.5 to 9.7 mol m^{-2} per day for red-leaf lettuce plants for indoor gardening systems [26]. A few studies explored the effect of DLI on the growth and development of green-leaved romaine lettuce in an indoor farming system.

Besides the measurements of morphological traits, biomass production, or sensory attributes, the effect of different growing conditions on lettuce growth and quality may also be evaluated using artificial intelligence approaches including machine learning [27–29]. It is desirable to increase the intelligence of machines. Machines can be trained using the available data for processing and analyzing visual data and making predictions without human intervention [30,31]. The application of artificial intelligence can be an important component of the agricultural revolution and can allow, for example, predicting crop yield or evaluating the plant quality using image processing [32]. The results obtained by models built using artificial intelligence can be accurate and promising [33]. The need to increase the efficiency in agricultural production and decrease the environmental burden, as well as increase in the demand for food, can involve the necessity for the application of techniques

of data processing with increased computational power in modern agriculture. These techniques can meet the growing demands of smart farming [34].

The objective of this study was to determine the optimal daily light integral (DLI) for romaine lettuce grown in an indoor vertical cultivation system by investigating biomass, morphology, and quality features important for consumers, such as the concentration of nitrates in leaves and sensory value. A novelty in our experiment was evaluating the differentiation of the samples, a discrimination analysis using texture features of leaf images and machine learning algorithms. Light-use efficiency is important for understanding biomass production, therefore, the evaluation of the quantum photosynthetic yield of photosystem II (PSII) was performed using the chlorophyll fluorescence method. Two levels of PPFD and two photoperiods corresponding to four DLIs in the range of 9–17 mol m⁻² per day were used in the study.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Two cultivars of romaine lettuce *Lactuca sativa* var. *longifolium* with different growth characteristics were selected in this study. Cultivar ‘Casual’ (Syngenta) represents midi romaine lettuce with medium-compact heads, and cv. ‘Elizium’ (Enza Zaden) is a mini type (Little Gem) cultivar with compact heavy heads. Plants were grown in the Indoor Controlled Environment Agriculture (CEA) facility (6.0 × 2.6 × 3.2 m³) fitted with two two-shelf racks, as described previously [19]. Fourteen-day-old seedlings produced in rockwool cubes were used in the study. Plants were grown in polystyrene boxes with a capacity of 20 liters, filled with hydroponic nutrient solution. There were six plants in each box, which were mounted on floating polystyrene rafts (24 plants per m²). A hydroponic nutrient solution containing macro- and microelements (mg L⁻¹) N-NO₃—153, N-NH₄—20, P—32, K—210, Ca—200, Mg—32, Fe—1.7, Mn—0.67, Zn—0.2, B—0.3, Cu—0.15, and Mo—0.04 was used in the study. The electrical conductivity (EC) of the nutrient solution was 2.1 mS cm⁻¹, and pH 5.7. The hydroponic nutrient solution was changed out completely once a week and was aerated, which allowed to maintain a constant dissolved oxygen concentration of 9 mg L⁻¹. The temperature in the CEA facility was set at 22 °C day/night, and the relative air humidity at 65%. The experiment was set up on 28 July 2021, and lasted 30 days.

2.2. Experimental Design

The experiment used a two-factorial design of PPFD × photoperiod. In the case of both cultivars of romaine lettuce, there were three containers for each of the four light treatments and, thus, 12 containers in total for each of the cultivars. Six plants of one cultivar with three replications and four treatments were selected. A total of 144 plants were used for the experiment.

2.3. Light Treatments

A factorial experiment with two light factors, i.e., photosynthetic photon flux density (PPFD) 160 and 240 μmol m⁻² s⁻¹ and photoperiod (16 and 20 h), was carried out. Daily Light Integral (DLI) values for these light treatments were 9.2, 11.5, 13.8, and 17.3 mol m⁻² day⁻¹. Each shelf in the CEA facility was fitted with panels with LEDs (Light Emitting Diodes) emitting red (R)—Hyper Red 660 nm (Osram Osconique P 30-30), blue (B)—Deep Blue 440 nm (Osram Osconique P 30-30), and white (W)—6500 K (Samsung CRI 80) with spectral composition RGB 70:10:20.

2.4. Data Collection and Analysis

Plants were harvested after 30 days of cultivation in the Indoor Controlled Environment Agriculture facility by cutting leaves above the collar. Morphological traits (fresh and dry weight, plant height and diameter, head circumference, the number of leaves per plant, the number of outer and inner leaves with tipburn, commercial values on a scale of 1 to 5

[1—the worst and 5—the best quality], and nitrate content) in leaves were determined for two genotypes. Nitrates were analyzed by the potentiometric method in six replicates.

2.5. Chlorophyll *a* Fluorescence of Light-Adapted Plants

The chlorophyll fluorescence of photosystem II (PSII) was measured with a Modulated Chlorophyll Fluorometer OS5p+ (Opti Sciences) using light-adapted measuring protocol Y(II). This test allows the measurement of the efficiency of photosystem II under actual light adapted environmental and physiological conditions and is an effective and sensitive way to measure plant samples under ambient or artificial lighting conditions. Quantum photosynthetic yield of PSII measurement of the efficiency with which absorbed light is used to drive photochemistry in the light-adapted state. Measurements were performed on the youngest fully developed leaf just before assessing morphological traits and plant harvesting. Nine plants for each of the light treatments were measured.

2.6. Statistical Analysis of Growth Traits

Two-way ANOVAs were used to test the effects of PPFD and photoperiod on the growth traits of romaine lettuce. The treatment means were compared using Tukey's HSD. Statistical analysis was performed using the STATISTICA software, version 13.1 (StatSoft Inc., Tulsa, OK, USA).

2.7. Sensory Analysis

2.7.1. Sensory Quality Attributes

For sensory evaluation, the method of Quantitative Description Analysis (QDA), i.e., sensory profiling, was used in accordance with the procedure included in the standard Sensory Profiling ISO 13299:2016. The assessment was carried out in the sensory laboratory of the National Institute of Horticulture Research in Skierniewice, Poland, meeting the requirements of the standard PN-ISO 8589 (Sensory analysis—General guidelines for designing a laboratory for sensory analysis). The evaluation was performed by a 10-person team of evaluation experts with many years of experience in the sensory evaluation of vegetables and fruit. The brainstorming session was run to select attributes. During the analysis, each person was in the individual evaluation box equipped with the computer and specialized software (ANALSENS ver. 7) designed for the preparation of tests, recording of individual assessments and processing of the results. Lettuce leaf samples were brought to the stands. For sensory evaluation of romaine lettuce, the following quality descriptors were selected: lettuce smell, color, crispness, juiciness, lettuce taste, sweet taste, bitter taste, grassy taste, and overall quality. Overall quality notes are based on notes for all sensory quality attributes and summarize the quality impression of the evaluated sample. The intensity of each descriptor was assessed on a graphical scale, corresponding to 0 (low intensity)–10 (high intensity) conventional units, with marginal markings. The evaluation was carried out in two sessions. In the case of each attribute, the mean was calculated.

2.7.2. Statistical Analysis of Sensory Quality Descriptors

Principal component analysis (PCA) is a widely used multivariate analytical statistical technique. It was performed to synthetically determine the similarities and differences in sensory quality of romaine lettuce using the STATISTICA software ver.13.1. PCA was applied to QDA data to reduce the set of dependent variables (i.e., attributes) to a smaller set of underlying variables (called factors) based on patterns of correlation among the original variables [35]. When performing PCA, lettuce smell, color, crispness, juiciness, lettuce taste, sweet taste, bitter taste, grassy taste, and overall quality were included.

2.8. Discrimination Based on Image Textures

2.8.1. Image Processing

The leaves of cv. 'Casual' and cv. 'Elizium' romaine lettuce cultivars were imaged using a designed system consisting of a digital camera and LED (Light Emitting Diode)

illumination with stable parameters. The digital camera and light source were placed in a box of dimensions 1 m × 1 m × 1 m with black internal walls. Color calibration of the digital camera was performed. The upper surface of each lettuce leaf was imaged separately. The leaves were placed on a black background. This procedure facilitated the segmentation of each image into a lighter leaf and black background based on pixel brightness intensity. Before the processing, the obtained images were converted to BMP format. The MaZda software (Łódź University of Technology, Institute of Electronics, Poland) [36] was used to process the leaf images. The images were converted to individual color channels $L, a, b, R, G, B, U, V, S, X, Y, Z$. The L^* is the lightness component, a^* —green (negative values) or red (positive values), b^* —blue (negative values) or yellow (positive values), R —red, B —blue, G —green, U and V determine the color itself (chromaticity), S —Saturation, Y —lightness, and X and Z components are color information [37,38]. For the leaf image in each channel, about 180 textures were computed. Thus, a total of approximately 2100 textural features were determined for one leaf image for all color channels. The image texture was a function of the spatial variation of the pixel brightness intensity. Textures can give information about the object structure and their quantitative analysis can provide insights into object quality [39,40]. Textures were computed based on the co-occurrence matrix (132 textures), run-length matrix (20 textures), Haar wavelet transform (10 textures), histogram (9 textures), gradient map (5 textures), and autoregressive model (5 textures) [36,40].

Textural features with the highest discriminative power were used to build the models for distinguishing the samples that differed in PPFD ($160 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $240 \mu\text{mol m}^{-2} \text{s}^{-1}$) and photoperiod (16 h and 20 h). A total of 80 cases were obtained including 20 cases for each type of sample. The obtained data were intended to analyze using artificial intelligence involving a machine learning approach.

2.8.2. Discriminant Analysis

A discriminant analysis was performed using the WEKA 3.9 machine learning software (Machine Learning Group, University of Waikato) [41–43]. Four classes of lettuce leaves were considered. The first step of the analysis included the textural attribute selection. The Best First search method and CFS (Correlation-based Feature Selection) Subset Evaluator were used. The leaf samples were discriminated using the 10-fold cross-validation mode. The models were developed based on image textures from all color channels $L, a, b, R, G, B, U, V, S, X, Y$, and Z . Different machine learning algorithms from the groups of Bayes, Functions, Lazy, Meta, Rules, and Trees were tested to select the one algorithm providing the highest discrimination performance metrics. The following metrics were determined: accuracy (Equation (1)), TPR—True Positive Rate (Equation (2)), FPR—False Positive Rate (Equation (3)), Precision (Equation (4)), F-Measure (Equation (5)), ROC Area—Receiver Operating Characteristic Area (Equation (6)), and PRC Area—Precision-Recall Area (Equation (7)).

$$\text{Accuracy} = (\text{TP} + \text{TN}) / (\text{TP} + \text{FP} + \text{TN} + \text{FN}) \times 100 \quad (1)$$

$$\text{TPR} = \text{Recall} = \text{TP} / (\text{TP} + \text{FN}) \quad (2)$$

$$\text{FPR} = \text{FP} / (\text{FP} + \text{TN}) \quad (3)$$

$$\text{Precision} = \text{TP} / (\text{TP} + \text{FP}) \quad (4)$$

$$\text{F-Measure} = 2\text{TP} / (2\text{TP} + \text{FP} + \text{FN}) \quad (5)$$

$$\text{ROC Area} = \text{Area Under TPR vs. FPR Curve} \quad (6)$$

$$\text{PRC Area} = \text{Area Under Precision vs. Recall Curve} \quad (7)$$

TP—True Positive, TN—True Negative, FP—False Positive, FN—False Negative.

3. Results

3.1. Plant Growth

The growth of romaine lettuce plants in the Indoor Controlled Environment Agriculture facility was significantly affected by the daily amount of light reaching the plants; however, there were some differences in plant growth between the two compared lettuce cultivars. For cv. 'Casual' (midi type Romaine lettuce), both increasing photosynthetic photon flux density (PPFD) from 160 to 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$, as well as extending the photoperiod from 16 to 20 h, stimulated the biomass production, however, photoperiod extension was more effective than increasing PPFD (30% vs. 26%). Plants grown for 30 days under higher PPFD (240 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and long photoperiod (20 h), which gives 17.3 mol m^{-2} daily light integral (DLI) per day, had the highest fresh (353.1 g) and dry weight of leaves (14.6 g) and the number of leaves per plant were the highest, while under lower PPFD (160 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and shorter photoperiod (16 h), which gives 9.2 mol m^{-2} (DLI) per day, had the smallest fresh (215.0 g) and dry mass (6.7 g), as well as the lowest number of leaves and smallest head circumference (Figure 1). Plant height and diameter of cv. 'Casual' plants were not affected by applied light conditions. Our results showed that the stimulation of biomass production as a result of increased DLI from 9.2 to 17.3 mol m^{-2} per day enhanced drying up of the leaf edges (tipburn), both external and internal, which, in turn, deteriorated the commercial value of lettuce. The highest number of outer and inner leaves with tipburn was observed under high PPFD and long photoperiod (17.3 mol m^{-2} DLI per day), respectively, 14 and 18% of all leaves, while under lower PPFD and shorter photoperiod (9.2 mol m^{-2} DLI per day), the numbers of tipburn leaves were 4% (outer) and 2% (inner).

For cv. 'Elizium' (mini type Romaine lettuce) increasing PPFD from 160 to 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ stimulated biomass production only at 16 h photoperiod and such a reaction was not noted with longer photoperiod (Figure 2). The highest fresh weight of leaves had the plants grown at longer photoperiod irrespective of PPFD as well as at 16 h photoperiod but only under higher PPFD (on average 239.3 g), i.e., from 11.5 to 17.3 mol m^{-2} DLI per day and the lowest at 16 h and under lower PPFD (191.3 g), i.e., at 9.2 mol m^{-2} DLI per day. Dry weight of leaves was the highest at 20 h photoperiod (on average 8.4 g) and the lowest at 16-h photoperiod and lower PPFD (5.4 g). Moreover, plants grown under higher PPFD were smaller and had a smaller diameter than those grown under lower PPFD, regardless of the length of the photoperiod. Increasing DLI from 9.2 to 17.3 mol m^{-2} per day increased the number of outer and inner leaves with symptoms of tipburn, similar to the cv. 'Casual'. The highest number of outer and inner leaves with tipburn was observed under high PPFD and long photoperiod, i.e., 17.3 mol m^{-2} DLI per day, respectively, 50 and 8% of all leaves, while under lower PPFD and 16 h photoperiod 9.2 mol m^{-2} DLI per day, the numbers of tipburn leaves were 4% (outer) and 0% (inner).

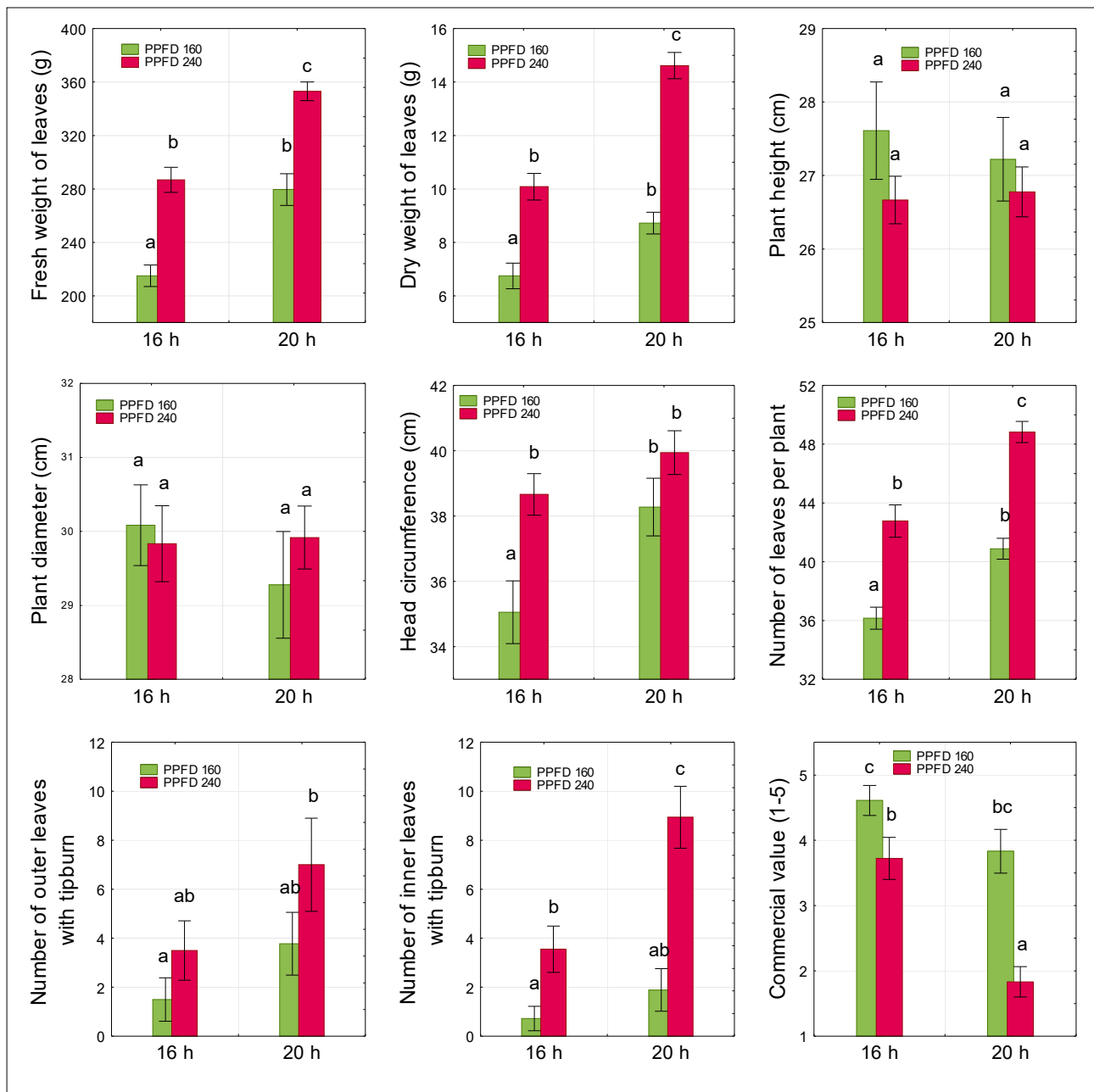


Figure 1. Fresh and dry weight of leaves, plant height, plant diameter, head circumference, number of leaves per plant, number of outer and inner leaves with tipburn, and commercial value of cv. ‘Casual’ romaine lettuce grown under different light environments (photoperiod 16 and 20 h and PPFD 160 and 240 μmol m⁻² s⁻¹). Bars represent means ± SE. Means followed by the same letter are not significantly different (*p* < 0.05) according to Tukey’s HSD test.

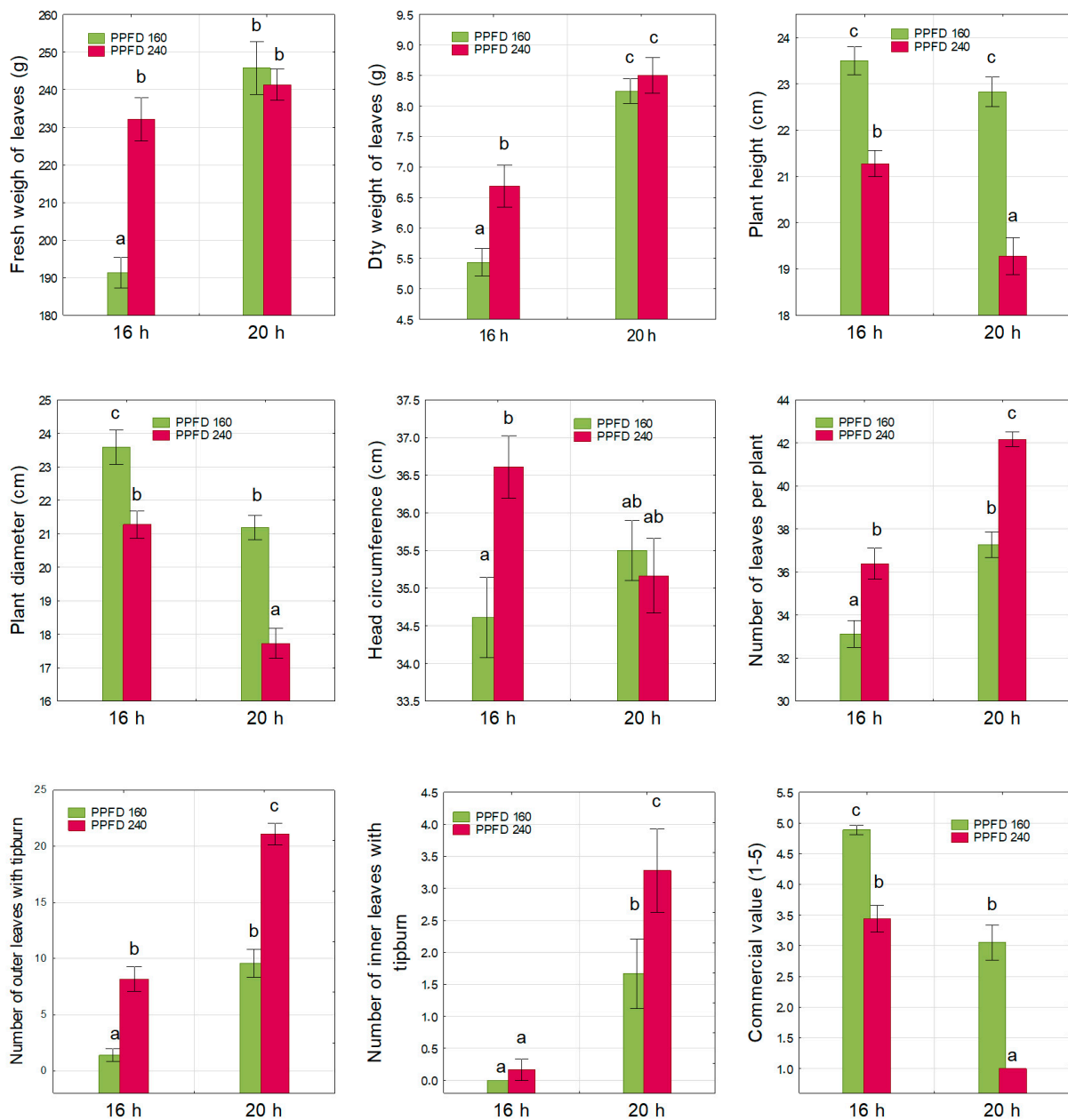


Figure 2. Fresh and dry weight of leaves, plant height, plant diameter, head circumference, number of leaves per plant, number of outer and inner leaves with tipburn, and commercial value of cv. 'Elizium' romaine lettuce grown under different light environments (photoperiod 16 and 20 h and PPFD 160 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Bars represent means \pm SE. Means followed by the same letter are not significantly different ($p < 0.05$) according to Tukey's HSD test.

3.2. Chlorophyll Fluorescence

Quantum Photosynthetic Yield of PSII (γ) in light-acclimated romaine lettuce cv. 'Casual' and cv. 'Elizium' was essentially stable (≈ 0.67) at 16 h photoperiod irrespective of PPFD (160 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$), as well as 20 h photoperiod and 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (DLI in the range from 9.2 to 13.8 $\text{mol m}^{-2} \text{day}^{-1}$) and significantly declined (≈ 0.63) in 20 h photoperiod and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (DLI 17.3 $\text{mol m}^{-2} \text{day}^{-1}$, Figure 3).

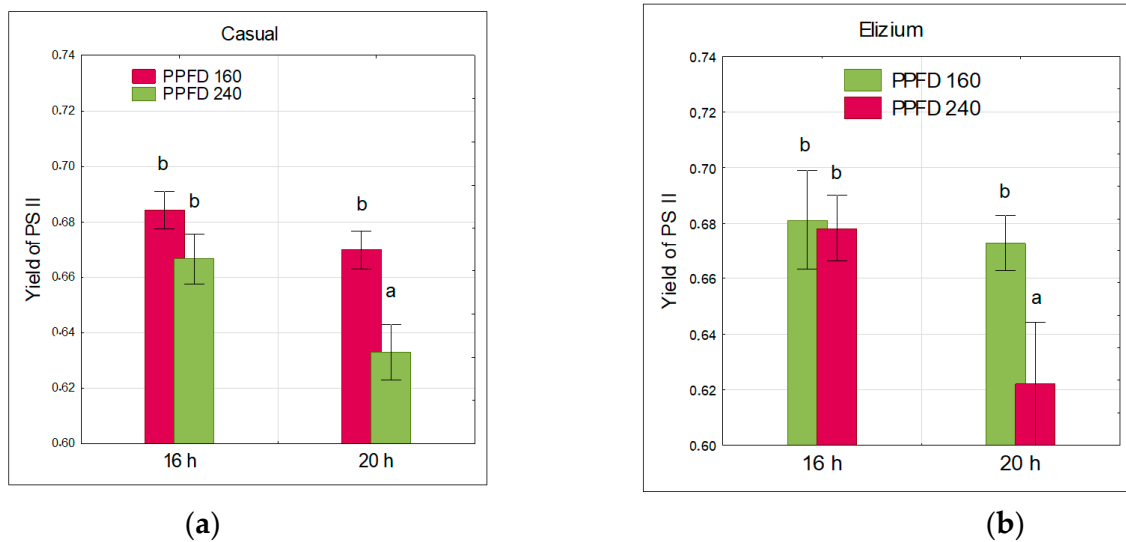


Figure 3. Quantum Photosynthetic Yield of PSII (Y) of cv. ‘Casual’ (a) and cv. ‘Elizium’ (b) romaine lettuce grown under different light environments (photoperiod 16 and 20 h and PPFD 160 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Bars represent means \pm SE. Means followed by the same letter are not significantly different ($p < 0.05$) according to Tukey’s HSD test.

3.3. Nitrate Nitrogen in Plants

Our study showed that low light conditions favor nitrate accumulation in romaine lettuce (Table 1). The highest nitrate content in leaves was noted at 16 h photoperiod and 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$, i.e., at the lowest DLI (9.2 mol m^{-2} per day) for both ‘Casual’ and ‘Elizium’ cultivars, 2094 and 1714 mg kg^{-1} f.w., respectively. Increasing DLI resulted in reduced nitrate nitrogen content in the leaves to the value of 1481 and 1218 mg kg^{-1} f.w. for cv. ‘Casual’ and cv. ‘Elizium’, respectively; however, there were no significant differences in nitrate content at DLI in the range 11.3 to 17.3 mol m^{-2} per day.

Table 1. Concentrations of nitrate nitrogen (mg kg^{-1} f.w.) in the leaves of cv. ‘Casual’ and cv. ‘Elizium’ romaine lettuce grown under different light environments (photoperiod 16 and 20 h and PPFD 160 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Bars represent means \pm SE. Means followed by the same letter are not significantly different ($p < 0.05$) according to Tukey’s HSD test.

Photoperiod/PPFD	Casual	Elizium
16/160	2094 \pm 171 b ¹	1714 \pm 54 b
16/240	1668 \pm 18 ab	1218 \pm 76 a
20/160	1481 \pm 201 a	1447 \pm 29 ab
20/240	1697 \pm 168 ab	1440 \pm 170 ab

¹ Means followed by the same letter for cultivar are not significantly different ($p < 0.05$) using Tukey’s HSD test. Means \pm SE of 6 plants.

3.4. Sensory Quality

QDA method results of sensory analysis for romaine lettuce in relation to light factors, i.e., PPFD (160 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and photoperiod (16 and 20 h), are shown in Figures 4 and 5. Sensory quality is a very important criterion for evaluating vegetables, determined by the purchasing preferences of consumers. In this study, the overall quality of romaine lettuce was scored on a significantly higher level after treatments PPFD 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$, regardless of cultivar and photoperiod time (Figure 4). Based on the mean values obtained from the overall quality assessment, it can be concluded that the cv. ‘Elizium’ lettuce cultivar compared to the cv. ‘Casual’ was characterized by slightly better sensory quality.

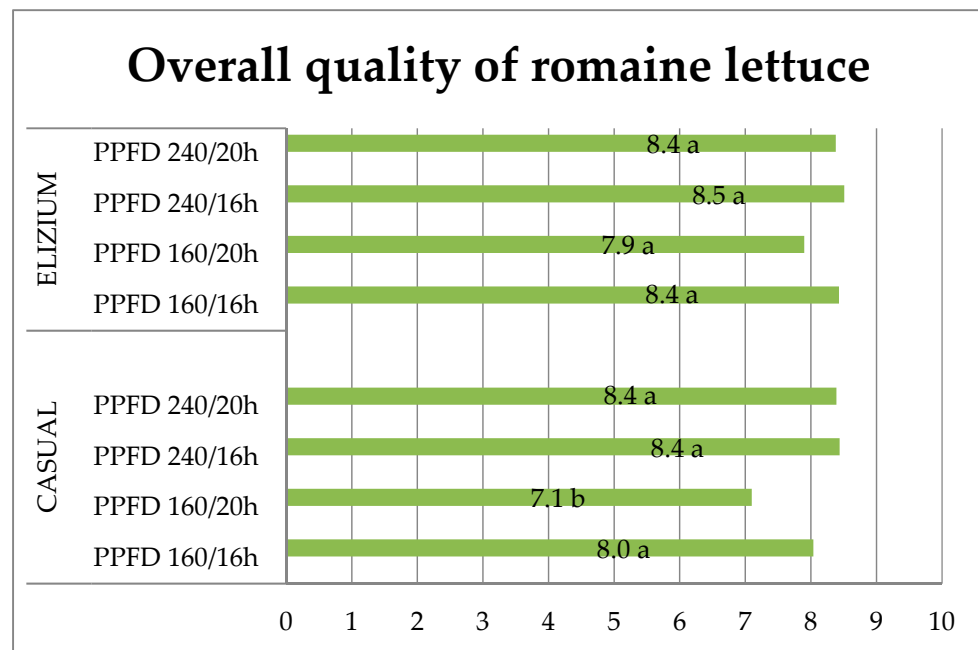


Figure 4. Comparison of the overall quality descriptor of romaine lettuce (scale 0–10 units). Means followed by the same letter are not significantly different ($p < 0.05$) according to Tukey’s HSD test.

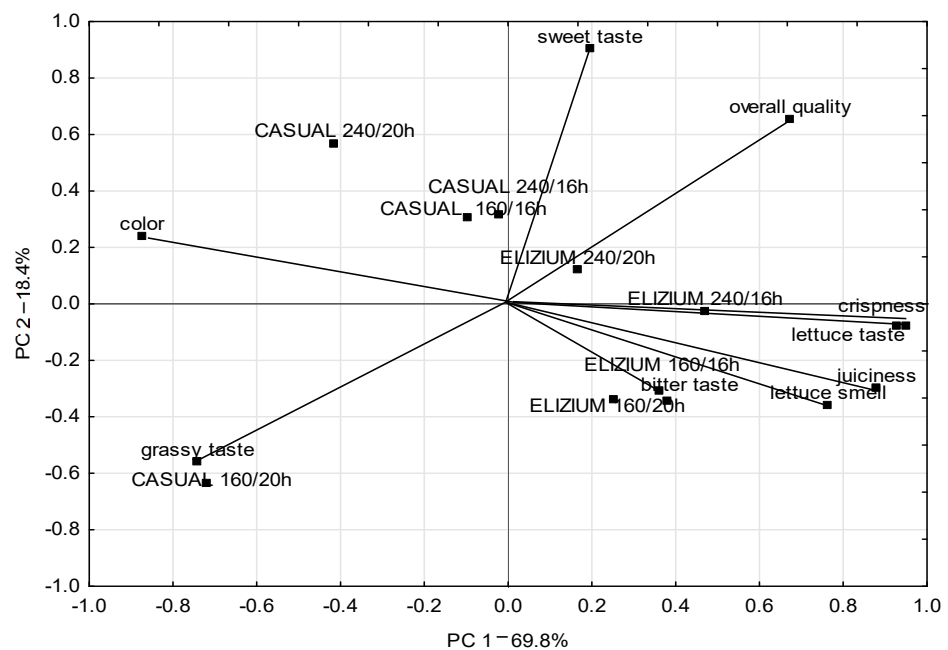


Figure 5. PCA biplot of similarities and differences in sensory profiles of romaine lettuce.

The similarities and statistical differences in the sensory quality of the evaluated romaine lettuce are shown in Figure 5. The space on the map was defined by the first two main components, explaining, respectively, 69.8% and 18.4% of the general variability. Overall quality was positively related to sweet taste, lettuce flavor and crispness (vectors follow the same direction). The cv. ‘Elizium’ lettuce of two subjects 240/20 h and 240/16 h was of the highest quality, which suggests a close location of the vector for assessing the overall quality and sweet taste. ‘Casual’ cultivar object 160/20 h was characterized by a lower sensory quality compared to the other objects, as evidenced by the location of this sample on the opposite side to the overall quality assessment vector and close to the grassy taste vector.

3.5. Lettuce Leaf Discrimination Based on Image Features

Among the tested machine learning algorithms, the Multilayer Perceptron from the group of Functions proved to be the most satisfactory in terms of performance metrics. Multilayer Perceptron is a type of neural network using the back-propagation method. A supervised learning technique was used [33]. It discriminated the leaves of lettuce subjected to different photoperiod and PPFD with satisfactory results in the case of both romaine lettuce cultivars ‘Casual’ and ‘Elizium’. The accuracy can range from 0 to 100%, and the TPR—True Positive Rate, FPR—False Positive Rate, Precision, F-Measure, ROC Area—Receiver Operating Characteristic Area, and PRC Area—Precision-Recall Area can be in the range of 0.000–1.000. The higher performance metrics such as accuracy, TPR, Precision, F-Measure, ROC Area, PRC Area, and the lower FPR, the more effective the model is [40]. The samples of cv. ‘Casual’ lettuce leaves were distinguished with an average accuracy of 98.75% (Table 2). The accuracy for three (16 h/160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (16/160), 16 h/240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (16/240), and 20 h/240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (20/240)) out of four classes reached 100%. Other performance metrics, namely, TPR, Precision, F-Measure, ROC Area, and PRC Area were equal to 1.000 and FPR was 0.000.

Table 2. The discrimination metrics of leaves of cv. ‘Casual’ romaine lettuce grown under different light environments (photoperiod 16 and 20 h and PPFD 160 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for the model built based on textures from all color channels of images using the Multilayer Perceptron machine learning algorithm.

Predicted Class (%)				Actual Class	Average Accuracy (%)	TPR	FPR	Precision	F-Measure	ROC Area	PRC Area
16/160	16/240	20/160	20/240								
100	0	0	0	16/160	98.75	1.000	0.000	1.000	1.000	1.000	1.000
0	100	0	0	16/240		1.000	0.000	1.000	1.000	1.000	1.000
0	0	95	5	20/160		0.950	0.000	1.000	0.974	0.997	0.992
0	0	0	100	20/240		1.000	0.017	0.952	0.976	0.998	0.995

TPR—True Positive Rate; FPR—False Positive Rate; ROC Area—Receiver Operating Characteristic Area; PRC Area—Precision-Recall Area.

In the case of lettuce grown under a photoperiod of 20 h and PPFD of 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (20/160), the accuracy was equal to 95%, and 5% of cases were incorrectly classified as lettuce subjected to 20 h and PPFD of 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (20/240). For samples of 20/160 and 20/240, other metrics, besides FPR (0.017), were greater than or equal to 0.950. The graphs shown in Figure 6 confirmed that the mean values of selected textures can be completely different for each class of lettuce leaves.

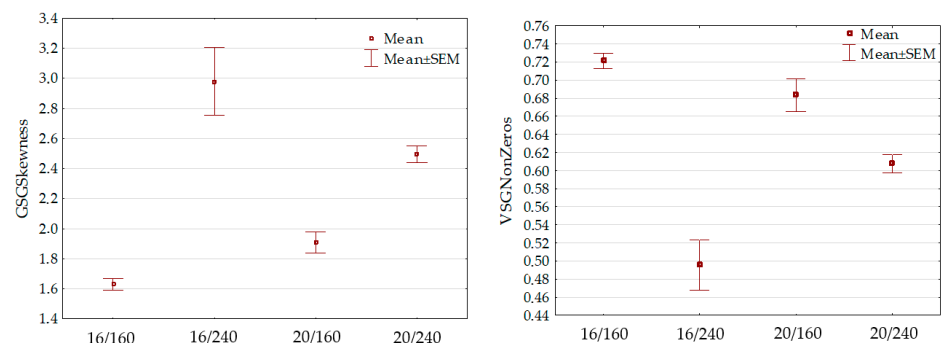


Figure 6. The values of selected image textures of the cv. ‘Casual’ lettuce leaves; SEM—standard error of the mean.

The average accuracy of discrimination of cv. ‘Elizium’ lettuce leaves from plants grown under different light environments was equal to 86.25% (Table 3) and was lower than for cv. ‘Casual’ (98.75%, Table 2). In the case of cv. ‘Elizium’ leaves (Table 3), none

of the classes was 100% different from the others in terms of image textures. The highest accuracy of 90% was observed for classes 16/160 and 20/240. As many as 10% of cases belonging to class ‘20/240’ were incorrectly included in class ‘16/240’. Furthermore, 5% of cases belonging to class ‘16/160’ were incorrectly classified as ‘20/240’, and the remaining 5% as ‘16/240’. The accuracies of 85 and 80% were obtained for the samples of ‘20/160’ and ‘16/240’, respectively. In the case of both classes ‘20/160’ and ‘16/240’, 10% of incorrectly classified cases were included in the class ‘16/160’. Five percent of cases belonging to class ‘16/240’ were classified as ‘20/160’, and 5% of ‘20/160’ leaves as ‘16/240’. Additionally, 5% of cases from the class ‘16/240’ were incorrectly included in the class ‘20/240’. Exemplary image textures of cv. ‘Elizium’ lettuce leaves are presented in Figure 7. The mean values may differ between some classes. However, the differentiation for cv. ‘Elizium’ is less visible (Figure 7) than for cv. ‘Casual’ (Figure 6).

Table 3. The discrimination metrics of leaves of cv. ‘Elizium’ romaine lettuce grown under different light environments (photoperiod 16 and 20 h and PPFD 160 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for the model built based on image textures from all color channels using the Multilayer Perceptron machine learning algorithm.

Predicted Class (%)				Actual Class	Average Accuracy (%)	TPR	FPR	Precision	F-Measure	ROC Area	PRC Area
16/160	16/240	20/160	20/240								
90	5	0	5	16/160	86.25	0.900	0.067	0.818	0.857	0.960	0.850
10	80	5	5	16/240		0.800	0.067	0.800	0.800	0.833	0.786
10	5	85	0	20/160		0.850	0.017	0.944	0.895	0.967	0.951
0	10	0	90	20/240		0.900	0.033	0.900	0.900	0.974	0.905

TPR—True Positive Rate; FPR—False Positive Rate; ROC Area—Receiver Operating Characteristic Area; PRC Area—Precision-Recall Area.

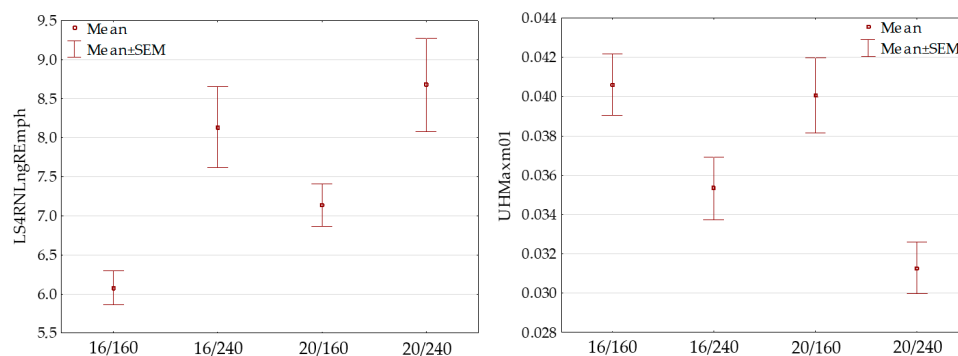


Figure 7. The values of selected image textures of the cv. ‘Elizium’ lettuce leaves; SEM—standard error of the mean.

4. Discussion

A comprehensive approach to evaluate the effect of different Daily Light Integral in an Indoor Controlled Environment on morphology, biomass production, sensory quality, and image textures of romaine lettuce is original and was not reported in the available literature. The use of different machine learning algorithms for the development of innovative discriminative models based on image textures of lettuce leaves subjected to different photoperiod and PPFD can also be considered as a great novelty of this study. Our research has shown that modification of the lighting environment enables the regulation of the growth rate of plants and influences the morphological traits of indoor cultivated romaine lettuce, as well as their quality characteristics important to consumers. As expected, the growth of the cv. ‘Casual’ (midi type lettuce) was faster than the cv. ‘Elizium’ (mini type lettuce); however, these cultivars had slightly different requirements for the amount of light reaching the plants. Improving the light conditions by increasing the daily light integral (DLI) in the range from 9.2 to 17.3 mol m^{-2} per day strongly stimulated the production of biomass of

cv. 'Casual' plants and a high yield (fresh plant weight of 350 g) was obtained within just 30 days. In the case of the cv. 'Elizium', increasing the DLI from 9.2 to 11.5 mol m⁻² per day by extending the day from 16 to 20 h and lower PPFD (160 μmol m⁻² s⁻¹), was sufficient to obtain a good yield (fresh plant weight of 240 g). Our results are consistent with earlier reports on leaf lettuce indicating the promotion of biomass production by increasing PPFD from 100 to 400 μmol m⁻² s⁻¹ [21] and from 150 to 300 μmol m⁻² s⁻¹ [44] and extending illumination time [2,45].

Quantum photosynthetic yield of PSII of romaine lettuce evaluated under actual light conditions, in light-adapted state PSII, showed no substantial difference in the range 9.2 to 13.8 mol m⁻² per day DLI and significantly decreased at the highest DLI (17.3 mol m⁻² per day). A similar relation was previously observed by Weaver and van Iersel [46]. The decrease in the yield of PSII can suggest increased thermal dissipation of absorbed light energy as a result of photoprotective processes and a lower amount of absorbed energy driving photochemistry [47] or alternative electron absorption such as reduction of nitrates [48].

Our research showed that the rapid growth rate of indoor cultivated romaine lettuce contributed to the disturbance of physiological processes as indicated by drying of the tops of the leaves. In the conditions where the highest yield of cv. 'Casual' lettuce was obtained, i.e., at the highest DLI, as much as 32% of leaves (both external and internal) had tipburn symptoms, which resulted in the poor visual quality of the lettuce. The share of leaves with tipburn in cv. 'Elizium' was as high as 57% at the highest DLI, and significantly lower (30 and 22%) at DLI 11.5 and 13.8 mol m⁻² per day, respectively. At the lowest DLI, the share of leaves with tipburn was the lowest for cv. 'Casual' (6%) and cv. 'Elizium' (4%), and their commercial value was very high. A strong relationship between the growth rate of romaine lettuce and the tipburn occurrence was previously demonstrated by Xu et al. [19]. One of the main causes of the physiological disturbances causing tipburn is insufficient supply of Ca (calcium) to young romaine lettuce leaves [44] and climatic conditions in indoor plant production systems favor tipburn occurrence [49].

There is a strong relationship between photosynthesis and nitrate assimilation in plants, as lettuce is a hyperaccumulator of nitrates and easily accumulates high nitrates in leaves [50]. A high light intensity can promote through increasing photosynthetic production and then nitrate accumulation. The highest total N content in lettuce was obtained under 450 μmol m⁻² s⁻¹ × 3/4 strength NSC (nutrient solution concentration) and the lowest—under 350 μmol m⁻² s⁻¹ × 1/2 strength NSC [51]. The nitrate content in lettuce leaves generally decreased with increasing PPFD [21,52], even when high levels of PPFD were used only at the end of the production period [53]. To protect human health, most European countries regulate the nitrate content in leafy vegetables [54]. The maximum limits for nitrates in lettuce are 5000 in winter-grown plants and 4000 mg per kg of fresh product in other seasons of the year. The results of our study showed that the concentration of nitrates in the leaves of cv. 'Casual' and cv. 'Elizium' romaine lettuce grown in the indoor controlled environment was the highest at the lower daily light integral, 2094 and 1714 mg kg⁻¹ f.w., respectively; however, it did not exceed the limits for greenhouse winter crops. The statistically significant effect of PPFD and photoperiod on the sensory quality of lettuce leaves was confirmed in the case of cv. 'Casual'. Despite the limited literature data on the sensory quality of romaine lettuce grown under different light conditions, for example, Lin et al. [12] included the sensory attributes in lettuce evaluation. The authors reported that the combined RBW LEDs resulted in many positive effects on the growth, development, nutrition, appearance, and edible quality of lettuce plants. The high scores for the shape, color, crispness, and sweet taste of Boston lettuce were influenced by the treatment of the RBW (red (R), blue (B), and white (W)) and FL (fluorescent lamp) plants. It was confirmed that light can influence the accumulation of sugars and the degradation of the nitrate level in plants. Therefore, the higher sugar level can result in a sweeter taste and higher acceptance by consumers, and the products with a lower nitrate level can be characterized by human health benefits [12]. Light can result in changes in phytochemicals, thereby affecting plant

taste and pigmentation. Light induction may cause the production of phenolic acids and flavonoids providing a protective mechanism against solar radiation [55].

The obtained results revealed differences in fresh and dry weight of leaves, plant height, plant diameter, head circumference, number of leaves per plant, number of outer and inner leaves with tipburn, commercial value, and overall quality between some samples of romaine lettuce. The presence of differences between romaine lettuce subjected to different photoperiod and PPFD was also confirmed by the objective and non-destructive evaluation using image features and machine learning algorithms. Some samples were distinguished from others with an accuracy of up to 100% as in the case of 16 h/160 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 16 h/240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 20 h/240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for cv. 'Casual' lettuce. Additionally, for 16 h/160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 16 h/240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ samples, the values of True Positive Rate, Precision, F-Measure, Receiver Operating Characteristic Area, and Precision-Recall Area reached 1.000 and False Positive Rate was equal to 0.000. The accuracy of 100%, low False Positive Rate, and high other metrics indicated an effective model. These results are very promising. Even more so, as the available literature reports many applications of image processing and artificial intelligence in lettuce research, and the information on romaine lettuce grown under different photoperiod and PPFD is missing. Digital image analysis was successively applied to real-time, nondestructive biomass assessments of lettuce cultivated in a greenhouse, high tunnel, and outdoor [56]. Spectral indices extracted from digital images enabled the nitrogen and chlorophyll estimation in romaine lettuce grown in high tunnels [57]. An image-based approach and mask region-based convolutional neural network (Mask R-CNN) model turned out to be useful for monitoring the growth rate of lettuce in a hydroponic system [58]. Convolutional neural networks were also useful for cultivar classification of lettuce based on image features [59]. Furthermore, abnormal hydroponic lettuce leaves were detected using image analysis and machine learning [60]. Furthermore, machine vision was applied for measurement of the fresh weight of lettuce in a closed hydroponic system [61], deep learning models (for the determination of nutrient concentration in hydroponically grown lettuce) [62], Backpropagation Neural Network and digital image processing (for determination of the healthiness of romaine lettuce) [63]. Machine learning may be promising due to better computational power than conventional techniques of data processing. The application of machine learning can extract more necessary information for the evaluation of plant quality [34]. Machine vision can have many advantages, such as high accuracy, high repeatability, and low costs. Therefore, inspection systems using machine vision can be applied in modern manufacturers, e.g., in food processing for quality control. Such systems can support making a decision besides techniques and methods involving, e.g., human experts, spectroscopy, or molecular markers, which may be time-consuming, subjective, or more expensive [64]. Due to the confirmation of the usefulness of image processing and artificial intelligence in our study for the evaluation of romaine lettuce grown under different light conditions in an indoor controlled environment, the research can continue and expand, e.g., by using artificial neural networks and deep learning as an addition to other manual measurements. The developed procedures can also be used for other romaine lettuce cultivars and other growing conditions.

5. Conclusions

This study has shown that the amount of light reaching the plants at DLI in the range of 9.2 to 17.3 mol m^{-2} per day significantly modifies the growth, photochemical processes, and quality of indoor cultivated romaine lettuce. Increasing the DLI increased biomass production, fresh weight, head circumference, and number of leaves, and reduced nitrate accumulation in the leaves, but, at the same time, deteriorated the visual quality of plant and quantum photosynthetic yield of PSII. However, the visual quality of the plants grown at the lowest PPFD 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 16 h photoperiod was the highest as leaves with tipburn appeared sporadically. Despite nitrate accumulation rinsing in plants grown at the lowest DLI, the level was far below the limit imposed for lettuce by European Community

Regulation. PPFD and photoperiod also influenced the sensory attributes of lettuce leaves. The ‘Elizium’ lettuce subjected to PPFD $240 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 16 h photoperiod was characterized by the highest overall quality. Whereas cv. ‘Casual’ lettuce grown under PPFD $160 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 20 h photoperiod revealed the lowest sensory quality. The application of leaf image processing and artificial intelligence allowed distinguishing the lettuce samples in terms of PPFD and photoperiod with a high probability. This approach is promising as it has shown that it is possible to evaluate the quality of the samples using objective and non-destructive techniques. Future research may extend the obtained results using artificial neural networks and deep learning. Furthermore, a significant benefit of using a lower PPFD and shorter daylength is reduced energy costs associated with electric lighting. These results could help improve romaine lettuce production in plant factories due to the indication of optimal light conditions.

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