Hydroponic NFT-Based Indoor Farming of Red and Green Lettuce Microgreens in Response to Artificial Lighting

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Abstract. The plant factory controls temperature, lighting, plant nutrients, and other environmental factors to provide a consistent, plant-friendly environment for predictable yields. In addition, microgreens can be grown profitably in expensive urban plant factories in a short harvest period. This study uses hydroponics with artificial lighting in the form of a Light-Emitting Diode (LED) with different ratios of lux and Photosynthetic Photon Flux Density (PPFD), i.e., 100 lx and 44.26 mol$\bullet$m$^{-2}\bullet$s^{-1} PPFD for 100% blue; 600 lx and 20.32 mol$\bullet$m$^{-2}\bullet$s^{-1} PPFD for 100% red; 1000 lx and 95.92 mol$\bullet$m$^{-2}\bullet$s^{-1} PPFD for 100% white, with maintained 25–27 °C of temperature and 70–80% of relative humidity. Microgreens of red and green lettuce were watered once a day with 250 ppm of plant nutrients using a commercial AB mixture and liquid organic fertilizer. The LED light, plant nutrient, and lettuce variety utilized in this study significantly affected the plant height, color parameters of L*, a, and b, and chlorophyll content of SPAD chlorophyll, chlorophyll a, chlorophyll b, and total chlorophyll content seven days after planting, as determined by the three-way MANOVA and Duncan test. A PCA model with two PCs has 88.0% of the explained variables for a comprehensive analysis of the LED data's variability.

Keywords: Plant factory · Hydroponic · Artificial Lighting · Indoor Farming · Lettuce Microgreens · NFT-based

1 Introduction

Lettuce microgreens are among the most popular and are widely used in salads and other nutritious foods. Lettuce is rich in fiber and low in calories, which is ideal for dieters. It also contains vitamin A, folate, C, K, and phenolic compounds [1]. Nutritional quality is frequently related to plant characteristics. Different leaf colors, such as antioxidant substances, might impact the nutritional value of a plant. Red lettuce has higher levels of lipophilic antioxidant activity, ascorbic acid, and phenolic compounds than other green vegetables. Other beneficial substances commonly found in lettuce include flavonoids,

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beta-carotene, and chlorophyll, which are crucial for enhancing the antioxidant and nutritional content of the diet [2].

Chlorophyll is recognized as a highly valuable bioactive compound that has earned great attention in recent years, coinciding with healthy lifestyle movements. Chlorophyll has antioxidant, anti-inflammatory, antimutagenic, and cancer-fighting properties. Two types of chlorophyll are present in lettuce: Chlorophyll a and Chlorophyll b [3–5]. Chlorophyll a and b function as photoreceptors that aid plants in absorbing and transmitting light energy [6]. The chlorophyll concentration in leafy vegetables is influenced by various factors, including nutrients and light. Chlorophyll is a crucial factor in plant development because it absorbs sunlight and uses its energy to produce organic compounds for photosynthesis [7]. Therefore, to grow plants indoors, artificial lighting must be used as a substitute for sunshine so that photosynthesis can occur. Plant factories are commonly employed for indoor farming as it allows us to regulate the environmental elements that affect the plants. There are a large number of different systems that are amenable to being modified for application in the plant factories sector. The most prevalent is the hydroponic system utilizing the Nutrient Film Technique (NFT). It aids in nutrient absorption by plant roots by diluting plant nutrients in the water and suspending them in a solution [8].

LEDs (light-emitting diodes) are frequently employed in indoor agriculture. Red and blue light benefit photosynthesis [9]. Red-colored LED lights are connected with plant height, exhibiting a strong correlation within six weeks of lettuce planting [10]. Blue light alters chlorophyll and chloroplast formation [11]. White LED light was crucial in apple plants' seedling stage [12]. Additionally, white LED affects the fresh weight of lettuce plants [13]. In addition to light, plants required nutrients for optimal productivity, which included plant elongation. Planting media must have a fair number of mineral components; hence, plant nutrients are usually utilized. Additional study indicates that the R:B ratio influences plant growth and photosynthesis. Despite substantial plant growth and quality research, the optimal light quality and R:B ratio has yet to be determined [14]. This phenomenon encourages investigation into the influence of the visible light spectrum on photosynthetic rate and plant growth to determine the impact of LED color and different nutrients on the development of red and green lettuce microgreens.

This research aimed to determine the reaction of red and green lettuce microgreens to artificial lighting and various nutrients. It is necessary to investigate how LEDs, plant nutrients, and plant variety interact with one another regarding their physicochemical properties. The study used a plant factory with LEDs that generated different light colors, whereas red, blue, and white LEDs. This research used two other plant nutrients, AB Mix and Liquid Organic Fertilizer. This research will contribute to developing an optimal approach for cultivating red and green lettuce microgreens in indoor farming.

2 Materials and Methods

2.1 Plant Materials

In this study, red leaf lettuce (*Lactuca sativa* var. *Crispa* L., Known You Seed®) and green leaf lettuce (*Lactuca sativa*, Known You Seed®) were utilized. Planting was conducted in an environmental conditions-controlled chamber for seven days. Temperature and relative humidity were monitored through environmental data logger monitoring, ranging from 24–26 °C, and relative humidity at 68–70%. Both lettuces were planted in Rockwool media and placed in a 24 × 19 cm tray with two replicate trays on each grow light. In planting holes, seeds were manually sown by hand, and the distance between each planting hole was 2 cm. Watering was done once per day with liquid organic fertilizer (POCNASA ®) and AB Mix (Nutrisiip ®). Three different LED lamps were used as grow lights. The first one was 100% white with 1000 lx and 95.92 mol•m^{-2}•s^{-1} PPFD with a wavelength of 550 nm; the second one was 100% red color with 600 lx, 20.32 mol•m^{-2}•s^{-1} PPFD, with a wavelength of 660 nm; and lastly, we used 100% blue with 100 lx, 44.26 mol•m^{-2}•s^{-1} PPFD, with a wavelength of 470 nm. PPFD, lux, and wavelength were measured using a PAR meter and quantum sensor (LICOR LI-250Q PAR), consisting of the LI-250 Light Meter and LI-190R Quantum Sensor, in addition to a light meter (Lutron LX-107).

2.2 Physical Characteristics Analysis

Plant height, fresh weight, and color parameter measurements were carried out using plant microgreen, which harvests seven days after planting. Observable stems on the Rockwool surface were randomly measured to quantify plant height samples by measuring 18 samples using a ruler. Green spinach and red lettuce microgreen were weighed by every 18 samples from each area on each tray using an analytical balance. Chromameter (Konica Minolta CR-400, photodiode sensor) was used to measure the color of green and red lettuce microgreen by producing the values of L, a, and b. Each of the three samples represented a tray region containing six distinct locations.

2.3 Chlorophyll Content Analysis

When both varieties of microgreens reached seven days after planting, red and green microgreens were randomly collected. Each planting tray's microgreens were harvested until their weight reached a maximum of 2.5 g for six sample replications. The chlorophyll concentration was determined by extracting the material from the obtained microgreens, diluted in acetone:ethanol (1:1 v/v) for 24 h at dark-room temperature. After twenty-four hours, the extracted material turned green due to the presence of leaf pigment extract solutions.

Optical Density was determined by measuring chlorophyll concentrations using an Ultraviolet-Visible Spectrophotometer (UV-Vis) (LW Scientific UV-200-RS) (OD). UV-Vis was utilized at wavelengths of 440 nm, 645 nm during the peak absorption of chlorophyll a, and 663 nm during the peak absorption of chlorophyll b [15, 16].

$$\text{Chla} = (13.7\text{xOD}_{663}) - (5.76\text{xOD}_{645}) \quad (1)$$

$$\text{Chlb} = (25.8\text{xOD}_{645}) - (7.7\text{xOD}_{633}) \quad (2)$$

$$\text{Chltot} = (20.0\text{xOD}_{645}) - (0.1\text{xOD}_{633}) \quad (3)$$

Chl a: chlorophyll a ($mg.L^{-1}$).
Chl b: chlorophyll b ($mg.L^{-1}$).
Chl tot: total chlorophyll ($mg.L^{-1}$).

This study also utilized a SPAD (Soil Plant Analysis Development) meter (SPAD-502 Plus – Konica Minolta) to assess the SPAD chlorophyll of red and green lettuce microgreen.

2.4 Statistical Analysis

Plant height, fresh weight, color parameters of L, a, and b, and chlorophyll content of SPAD chlorophyll, chlorophyll a, chlorophyll b, and total chlorophyll obtained were analyzed by conducting three-way multivariate analysis of variance (MANOVA) to determine how LED and nutrients used are affecting the growth and physicochemical properties of red and green lettuce microgreen. Duncan's multiple range test (DMRT) was also used to further statistical comparison between the various LEDs (p 0.05).

In addition, the analytical method known as Principal Component Analysis (PCA) was applied to red and green lettuce microgreens to determine the relationship between plant growth and the physiochemical properties, reduce the dimension of the data, and identify patterns that were distinct between the various LEDs and the plant nutrients. This PCA was analyzed so that the results could be presented more concisely. The principal component analysis (PCA) involves the development of axes through the linear combination of data based on several elements known as principal components (PC), which are data predictions based on the variance that has the most significant impact. In addition, the PCs, which have more essential characteristics, will have a broader range of values and more pertinent information [17].

3 Result and Discussion

3.1 LEDs and Plant Nutrient Effect on Chlorophyll and Growth of Red and Green Lettuce

The three-way MANOVA test was used to compare the effects of different LED light colors and plant nutrients on plant height, fresh weight, color parameter (L, a, b value), chlorophyll SPAD, chlorophyll a, chlorophyll b, and total chlorophyll content, all of which are indicative of plant growth and physicochemical parameters of red and green lettuce microgreen. The Duncan test was also used to determine the effect of LED lighting on plant growth and the physicochemical parameters of red and green lettuce microgreens (Table 1).

Light is known to be an essential component of photosynthesis in plants. Chlorophyll is a photosynthetic pigment that aids plants in increasing metabolite production, hence enhancing photosynthesis activity [18]. Different chlorophyll concentrations are produced by the LEDs employed in this investigation. The white LED had the most significant impact on the chlorophyll content, producing the most excellent chlorophyll. However, chlorophyll contents acquired through SPAD analysis continue to be greater than those obtained from UV-VIS analysis and result alignment. Microgreens of red and green lettuce grown under blue LED have the second-highest chlorophyll concentration after those produced under white LED. In contrast, red LED produces the lowest consequence. Microgreens of red and green lettuce grown under white LED weigh somewhat more than those produced under red LED. The fresh weight significantly rises when exposed to red light in Rapeseed plants, indicating that the two phenomena are related [18]. In addition, the plant grown under red LED was the tallest, according to the data. Red light is known to trigger photosynthesis, whereas blue light is known to reduce leaf area and elongation while increasing photosynthetic efficiency per unit of leaf area. Similar photosynthetic rates are seen under both blue and white LEDs, causing both to yield the highest chlorophyll concentration ([16; 17]. When exposed to the red region of the visible spectrum, chlorophyll is known to absorb light actively [18]. Naturally, photosynthetic activity rises as a plant absorbs light, resulting in optimal plant growth.

Besides lighting, plant nutrient also plays a significant role in plant growth. The two nutrients used in this study, AB Mix nutrient, indicated a better result in the average growth parameter than liquid organic fertilizer. Other research showed that after 30 and 35 days after seeding, AB Mix used in lettuce plants is reported to grow longer in height than lettuce plants grown with liquid organic fertilizer [21]. In addition, further research revealed that using liquid organic fertilizer alone did not affect the plant height and the number of leaves on Pak Choy plants [22].

The color values of L, a, b produced by green and red lettuce microgreen resulted in the opposite trend of differences in chlorophyll content in various LED lights. In contrast with chlorophyll content, the white and blue LED has no distinction of color parameters and lower value than the red LED. The color values of L, a, b produced by green and red lettuce microgreen resulted in the opposite trend of differences in chlorophyll content in various LED lights. In contrast with chlorophyll content, the white and blue LED has.

no distinction of color parameters and lower value than the red LED. Other research reported that the chlorophyll content of various lettuce varieties is different and has no correlation to L, a, and b value [1]. Generally, this research indicated that the chlorophyll content of green lettuce microgreen was higher than red lettuce microgreen. Bevly et al. (2018) [23] reported that the red leaf lettuce plant didn't show a very excellent chlorophyll concentration, which was affected by anthocyanin content. The chlorophyll also showed a strong relationship with carotenoid attention simultaneously. It means the rise in carotenoid concentration on the leaf will be followed by total chlorophyll content. Chlorophyll contains excellent vitamins A, C, E, K, and carotene.

3.2 Relationship Between LEDs and Plant Nutrient Effect on Physicochemical Properties of Red and Green Lettuce

Figure 1 demonstrates the Principal Components of Analysis (PCA) of red and green lettuce microgreens towards their growth parameter such as plant height, SPAD, L*, a, b, chlorophyll a, chlorophyll b, and total chlorophyll content. The cumulative sum of the first two PCs is quantified by 88% of the total variance, with PC1 being 78% and PC2 10%. Samples are divided by varied LED as seen in Fig. 1.a based on PC1. The score plot PC1 vs. PC2 displayed that the score for red and green lettuce microgreens grown under the red LED is separated by PC1 with the blue and white ones. Scores

Table 1. The harvest quality of red and green lettuce microgreens after seven days of planting under different LED colors and plant nutrient.

LED Light	Plant Variety	Planting Media	Plant Height	Fresh Weight	SPAD	L*	a	b	Chlorophyll Content		
									a	b	total
			(**)	(***)	(**)	(*)	(*)	(*)	(****)	(**)	(****)
White	Red Lettuce	AB Mix	2.62 ± 0.46	0.0416 ± 0.0086	15.26 ± 2.17	52.5 ± 1.52	-13.5 ± 0.8	24.13 ± 1.64	13.36 ± 0.37	7.21 ± 0.66	13.17 ± 0.72
		POC	2.40 ± 0.36	0.0349 ± 0.0060	18.78 ± 1.91	51.0 ± 0.87	-13.07 ± 0.6	22.09 ± 1.11	15.18 ± 1.31	7.64 ± 0.49	14.47 ± 1.07
	Green Lettuce	AB Mix	3.28 ± 0.25	0.0272 ± 0.0061	13.96 ± 1.17	53.22 ± 1.54	-14.06 ± 1.14	25.63 ± 1.98	12.64 ± 0.69	6.79 ± 0.76	12.43 ± 0.92
		POC	2.92 ± 0.48	0.0272 ± 0.0056	15.88 ± 1.44	50.81 ± 1.28	-12.66 ± 0.90	21.4 ± 4.97	14.23 ± 1.03	8.05 ± 0.78	14.36 ± 1.09
Red	Red Lettuce	AB Mix	3.97 ± 0.37	0.0328 ± 0.0056	7.87 ± 1.84	62.13 ± 7.05	-13.78 ± 1.12	26.44 ± 1.91	6.29 ± 1.55	5.10 ± 0.66	7.72 ± 1.34
		POC	4.31 ± 0.28	0.0276 ± 0.0059	9.81 ± 2.32	56.83 ± 1.97	-13.11 ± 1.55	25.11 ± 2.71	6.12 ± 0.23	6.14 ± 1.05	7.46 ± 0.22
	Green Lettuce	AB Mix	4.46 ± 0.47	0.0273 ± 0.0056	9.78 ± 1.61	57.57 ± 1.99	-12.93 ± 1.18	24.69 ± 2.18	5.44 ± 0.56	4.83 ± 0.4	7.04 ± 0.63
		POC	4.21 ± 0.63	0.0272 ± 0.0059	9.46 ± 1.79	57.82 ± 1.5	-13.48 ± 0.79	25.86 ± 1.4	5.94 ± 0.98	5.04 ± 0.33	7.48 ± 0.75
Blue	Red Lettuce	AB Mix	1.74 ± 0.13	0.0335 ± 0.0067	16.04 ± 2.59	52.44 ± 1.17	-13.57 ± 0.72	24.2 ± 1.48	13.54 ± 0.78	7.73 ± 0.63	13.73 ± 0.84
		POC	1.34 ± 0.26	0.0209 ± 0.0052	18.75 ± 1.71	50.99 ± 0.99	-12.77 ± 0.81	21.69 ± 1.4	13.79 ± 1.47	6.14 ± 1.05	12.44 ± 0.33
	Green Lettuce	AB Mix	1.78 ± 0.09	0.0299 ± 0.0060	16.66 ± 1.82	51.74 ± 1.79	-13.54 ± 1.11	23.99 ± 2.04	15.46 ± 0.73	8.13 ± 1.67	15.05 ± 1.61
		POC	1.32 ± 0.19	0.0721 ± 0.1069	18.01 ± 1.85	50.12 ± 1.48	-12.41 ± 1.06	21.32 ± 1.79	15.13 ± 0.76	7.92 ± 1.09	14.69 ± 1.28
White			2.80 ± 0.51b	0.4078 ± 0.0315a	15.97 ± 2.45b	51.91 ± 1.65a	-13.32 ± 1.01a	23.31 ± 3.26a	13.85 ± 1.29b	7.42 ± 0.8b	13.61 ± 1.24b
Red			4.32 ± 0.48c	0.0287 ± 0.0061a	9.23 ± 2.03a	58.59 ± 4.32b	-13.32 ± 1.21a	25.52 ± 2.17b	5.95 ± 0.96a	4.97 ± 0.42a	7.43 ± 0.82a
Blue			1.54 ± 0.28a	0.0391 ± 0.0561a	17.36 ± 2.25c	51.32 ± 1.62a	-13.07 ± 1.047a	22.80 ± 2.12a	14.48 ± 1.25c	7.48 ± 1.35b	13.98 ± 1.47b

[*] Combining the factors between varied LED, plant nutrient, and plant variety determined the result of L, a, and b significantly, ** Combining the factors between plant nutrient and plant variety, or LED and plant variety, or LED and plant nutrient determined the result of SPAD, plant height, and chlorophyll b significantly, *** Combining the factors between plant nutrient and variety determined the result of fresh weigh significantly, *** * Combining the factors between LED and plant variety or LED and plant nutrient determined the result of leaf area, chlorophyll a and total chlorophyll content significantly, [a, b, c] showed that each different letter were significantly different between varied LED using Duncan test.

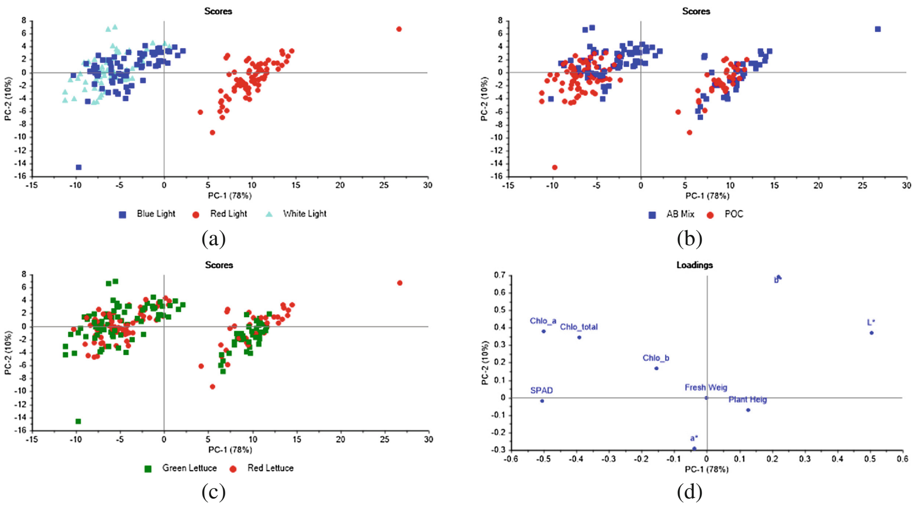
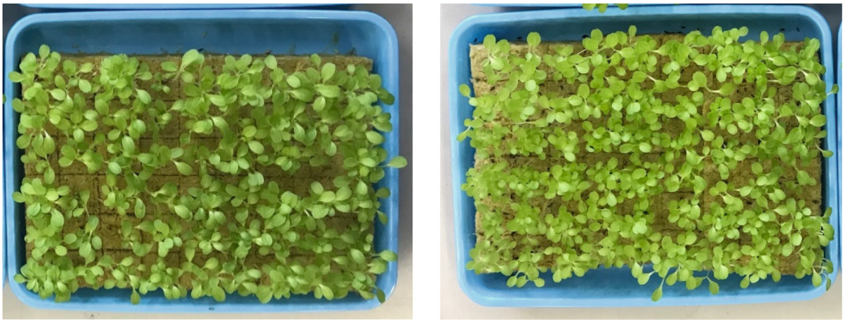


Fig. 1. Principal component analysis scores plot of different LED light (a), plant nutrient (b), lettuce microgreen variety (c), and loadings plot (d).

for microgreens under the red LED spread along the PC1 positive axis; meanwhile, the white and blue LED is on the PC1 negative side. Figure 1.b and Fig. 1.c exhibits the samples being clustered based on plant nutrient variety. However, the result has shown that plant variety and nutrients cannot characterize red and green lettuce microgreens.

The score plot in Fig. 1.a proves that red and green lettuce microgreens can be clustered by varied LED based on PC1. Based on the loading plot shown in Fig. 1.d, each parameter affects red and green lettuce microgreens differently. A PC score further from zero significantly influences the clustering of red and green lettuce microgreens. Plant height, L*, and b* values account for the positive PC1 score; meanwhile, SPAD, chlorophyll a, chlorophyll b, and total chlorophyll content are negative. Fresh weight has zero PC value, implying that it does not affect clustering.

The L* value has the highest positive impact based on PC1; meanwhile, SPAD and chlorophyll a have the most negative effect on plant growth. Conclusively, chlorophyll a will decrease, followed by the increase of the L* value. Based on the research done by Hu (2010) [24], Lab* of barley plants has a significant correlation with chlorophyll content and SPAD. It is possible because chlorophyll, carotenoid, and other pigments are strongly influenced by leaf color. Chlorophyll and carotenoids are responsible for the green and yellow colors in the leaf, respectively, the leaf color will appear brighter, and the L* value will increase. From the result that can be seen in Fig. 1.a, red has the most impact on the parameter. Other research reported that plants grown under red LEDs significantly displayed a lower growth value than white, green, blue, and red LEDs [25]. The DMRT result in Table 1. Also showed a similar outcome, where the L* value in the red LED is higher than the other LEDs. Due to their similarity, it is challenging to classify red and green lettuce microgreens based on their plant nutrients and varieties. Figure 1.d demonstrates that the L* value significantly benefits clustering, although it was unable to cluster the red and green lettuce microgreens in our study. The explanation



(a) (b)

Fig. 2. Microgreens are grown under white LED on the 7th day after sowing green leaf lettuce (a) and red leaf lettuce microgreens (b).

for this outcome is the slight difference between the values of L*, a, and b. Figure 2 demonstrates that both microgreens grown under all conditions have green leaves, as they are in the early stages of development. The lettuce plant's leaves will turn red eleven days after planting [26].

4 Conclusion

The various plant variety generate different chlorophyll levels, with the green lettuce microgreens containing more chlorophyll than the red ones. In this study, AB Mix and liquid organic fertilizer are utilized as nutrients; nonetheless, AB Mix produced superior results for the average growth parameter. The DMRT test indicates that red LED is ideal for promoting plant heights. Meanwhile, blue LED is suitable for increasing chlorophyll content, white LED is responsible for the fresh weight of the plant. PCA analysis shows that the application of two PCs explains 88% of the total variable; samples of red and green lettuce microgreens are classified by the various LEDs utilized in this study using PC1. This study reveals that artificial lighting is a feasible replacement for natural light.

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