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Article

The Qualitative and Quantitative Relationship of Lettuce Grown in Soilless Systems in a Mediterranean Greenhouse

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Abstract

This study evaluated the qualitative and quantitative performance of lettuce (cv. Romana) grown using different cultivation systems under Mediterranean greenhouse conditions equipped with photoluminescent glass panels. Five systems were compared: outdoor soil (PSO), indoor soil (PSI), aeroponic (A), hydroponic with inorganic nutrients (HSN), and hydroponic with organic nutrients (HSO). Morphological, physiological, and quality parameters were measured alongside solar irradiance and extended PAR. The results showed that aeroponics significantly outperformed other systems in fresh weight (52.7 g), photosynthetic pigments, and carotenoids, while HSO showed the lowest yield and quality. Although PSO had the highest antioxidant activity and phenolic content, it exhibited poor yield due to lower water use efficiency and light-induced stress. The PCA analysis highlighted distinct groupings among systems, with A linked to yield and pigment concentration, and PSO associated with antioxidant traits. Despite a 44.8% reduction in solar radiation inside the greenhouse, soilless systems—especially aeroponics—proved effective for maintaining high productivity and quality. These findings support the integration of soilless systems and photoluminescent technologies as sustainable strategies for high-efficiency lettuce production in controlled environments.

Keywords: soilless systems; indoor cultivations; lettuce quality; greenhouse; photoluminescent panels

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1. Introduction

The world population growth is expected to hit the estimated 11 billion people by 2100. Climate change has raised the issue of food scarcity and environmental pollution, along with an increasing pressure on natural resource utilisation through an urgent global food, land, water, and energy demand [1–3]. Currently, conventional soil-based agriculture is going towards different challenges concerning the depleted soil productivity and fertility. Other threats regard the rising of temperatures, frequent dry periods, and the unpredictability of weather conditions. This management leads to the overuse of water, soil, and their pollution [4]. Therefore, conventional agriculture can be complemented by modern forms of farming that take into account the reduction of natural resources used [5]. These facts have boosted researchers on broad studies about food security using technologies such as greenhouses [3]. Greenhouses are constructions that allow plants to grow in off-season periods by the control of an internal microclimate. In recent decades, to address the problem of climate change, the design of greenhouses has been improved to achieve more sustainable agricultural production [6,7]. A new and innovative food production system concerns the controlled environment agriculture in greenhouses in which technology is needed and produces a large amount of food with less input [8]. Protected crops in the Mediterranean area have expanded rapidly, and greenhouses are characterised by their low use of energy input [9]. Conversely, in northern Europe, the greenhouse environment has been optimised to achieve potential crop production through advanced computerised climate control systems and soilless cultivation [10].

Recently, plant production in indoor greenhouses has gained attention for growing leafy vegetables. Indeed, in several parts of the world, vegetable production systems were shifted from soil to soilless cultivation for their unique advantages [11,12]. Aeroponics, hydroponics, and aquaponics belong to the soilless cultivations and are considered one of the more sustainable and innovative agriculture approaches to quali–quantitively improve food production [1]. Soilless systems are suitable to produce crops with short cycles and high plant density. Inputs, size, locations, environmental conditions, and productivity can have a wide variation among soil and soilless systems [13,14]. Water supply, light, and plant nutrition management play a crucial role that is associated with productivity, biomass, and quality of crops [12]. Despite their initially high capital costs [15], soilless cultivation systems offer several advantages, including reduced water and pesticide use, a lower incidence of plant diseases, and the recirculation of nutrient solutions, which helps minimise environmental pollution [11,16]. Research for soilless systems was widely spread in plant physiology and productivity field in conventional greenhouses; thus, numerous vegetable crops were growing, such as lettuce, melon, tomato, herbs, and cucumber [1]. As mentioned above, the literature has widely demonstrated that the use of soilless systems, such as aeroponics and hydroponics in greenhouses, and allow for increased plant growth and quality. One of the primary leafy vegetables that increased to be marketed was lettuce. Traditionally, lettuce was cultivated in soil, but recently, soilless cultivation was taken in account, and higher yield and quality were achieved [4,13]. Leaf dry matter content and firmness were increased in soilless baby-leaf lettuce [17]. Gonnella et al. [18] evaluated the yield and quality of three multi-leaf lettuce in autumn and spring seasons by comparing soilless and soil cultivation and obtained an increase in crop yield of about 20% in soilless cultivation. Liška et al. [19] compared the yield and quality of lettuce in two soilless systems: aeroponic and aquaponic systems. Findings have shown that aeroponic system-grown lettuce had a better performance concerning the fresh weight of its head and roots. Our objective is to evaluate the impact of different soilless methods on the production and quality performance of lettuce grown inside the greenhouse (indoor environment) compared to an open field (outdoor environment), focusing on both biomass yield and the quality of the edible parts of lettuce across varying growth scenarios. The experiment was conducted in an automated glass greenhouse in a Mediterranean environment. The greenhouse glass was equipped with photoluminescent bands designed to shift incoming UV and green light into red and blue wavelengths to enhance photosynthetic efficiency [20]. Unlike full-cover spectral-shifting films, such as polyethylene (PE) or PMMA doped with rare-earth elements, which alter the entire light environment, photoluminescent bands offer localised spectral modification with minimal shading. This allows for better light penetration and temperature control while maintaining the benefits of spectral tuning. Comparative research is still limited, but preliminary findings suggest that photoluminescent bands may represent a more energy-efficient and crop-specific alternative to broad-coverage films [21]. In addition, it is also important to note that research has extensively tested the application of greenhouse covering films on the production performance of plants. Some of these have recently been experimented, and their function is to modify the light spectrum inside greenhouses to promote vegetative growth and the quality of plants [22]. Light is an important factor regulating plant growth; therefore, this study integrated measurements on solar irradiance and e-PPFD inside the greenhouse covered with photoluminescent bands and outside the greenhouse for the sole purpose of providing information on the lighting conditions of the experimental location. PAR is defined as the photosynthetically active radiation in the visible spectrum between 400 and 700 nm useful for plants grow. The e-PAR, includes the far-red photons in an extended spectrum from 400 to 750 nm [23]. A brief framework on how photo-selective materials affect plant development through light quality is also given.

These films proposed by the scientists, involve the use of polymers doped with photoluminescent materials, which convert the wavelengths from ultraviolet to red or blue and from green to red. They are divided into fluorescent dyes and organic and inorganic rare earths. The latter have been emphasised for their low cost and resistance to high temperatures [24]. Several studies have been carried out to compare the productive and qualitative performances of different plants under photo-conversion materials. Gao et al. [25] compared the production of sweet peppers under a polyolefin film doped with rare earths and un-doped (control), and they found that the fruit quality has shown improvement of 14. 2% along with a photosynthetic rate in the doped film rather than in the control. Paradiso et al. [26] compared under poly-methyl-methacrylate (PMMA)-based panels doped with 3% and 7% of rare ion elements (Europium and Disprosium) the production of rocket along with un-doped and white-washed panels. The rocket grown under 3% doped PMMA panels have shown the higher yield and antioxidant capacity with respect to others. Furthermore, in an experiment on Swiss chard, the total body biomass was 1.4 fold larger under a photo-conversion film than the control [27]. In tomato grown under a photo-conversion film, Yanykin et al. [28] found that there was a higher acceleration in plant growth and photochemical efficiency compared to the control film. In the study by Conti et al. [29], aubergine grown under a UV-to-red photo-conversion film showed a 19% increase in yield compared to a polyethylene (PE) film. In contrast, strawberries had the opposite trend to aubergine, with the highest yield under PE film. Magnani et al. [30] compared the yield and quality of lettuce and rocket cultivated in a hydroponic system under a photo-conversion film. They used two PE photo-selective films, one red and the other yellow, and a control PE film. They found that for lettuce, the yield increased in both films rather than the control, and dry matter increased in the red film.

2. Materials and Methods

2.1. Location, Plant Material, and Experimental Set-Up

The experiment was carried out in spring in an automated greenhouse located within the Alessandro Volta High School courtyard of Reggio Calabria (38°05′41″ N–15°39′39″ E) in Italy for 21 days, from 10 May to 31 May 2024. The greenhouse, measuring 6 × 3 m, is oriented northeast and constructed with a glass covering and an aluminium frame structure. The indoor equipment consists of aluminium racks to support the aeroponic system, aeroponic trays to support crops, two water tanks with water recirculation system, pipes for irrigating the different compartments of the aeroponics, air and irrigation pumps, a control unit that manages the fertiliser controller, the irrigation time, the water chiller, and LED (Light Emitting Diodes) lighting system (SERRANOVA s.r.l., Perugia, Italy) (Supplementary Figure S1). Lettuce seedling (*Lactuca sativa* L.) cv. Romana of 6 cm height were bought in black polyethylene trays; soil was washed from the roots, and the seedlings were transferred to different growth systems: aeroponic (A), outdoor potted soil lettuce (open-field PSO), indoor potted soil lettuce (inside the greenhouse PSI), hydroponic standard solution (with commercial nutrient solution inside the greenhouse HSN), and organic hydroponic (with organic nutrient liquid solution inside the greenhouse HSO). The experimental design was completely randomised, in which each system consisted of at least 6 replicates. PSO (outdoor) and PSI (indoor) potted growing systems were considered as negative and positive control treatments, respectively.

2.2. Growing Conditions

Commercial soil (Flortis peat 60%, green composted amendment 40%, pH in water 7.5, EC 0.70 dS/m, Orvital S.p.A, Milan, Italy) was used to grow lettuce for the PSO and PSI. For the PSO, PSI, A, and HSN growing systems, a commercial NPK fertiliser (PLANTAFOL® 20-20-20, Valagro S.p.A, Atessa, Italy) with 0.5 g/L dose dissolved in water was utilised to irrigate these systems, whereas a commercial organic NPK fertiliser (3-1-5, Pro Organic Grow with 2 mL dose, Terra Aquatica, Fleurance, France) was used for HSO growing system. During the growing period until harvest, for each system, the following operations were performed: PSO and PSI systems were irrigated with 50 mL nutrient solution/day/plant, whereas the nutrient solution for HSO and HSN systems was changed every week; for A system, each hour, the nozzles of irrigation system sprayed the nutrient solution for 2 min.

2.3. Morphological and Physiological Measurements

For each system, a time course of the leaf width (Lw, cm) and plant height (PH, cm) and number of true leaves (NLT) was measured.

During the vegetative phase, the physiological parameters such as water use efficiency (WUE, μmol CO_2μ mol^{-1} H_2O), transpiration rate (TrspRa, μmol H_2O $m^{-2}s^{-1}$), stomatal conductance (StoCon, mmol H_2O $m^{-2}s^{-1}$), and photosynthetic rate (PhsR, μmol CO_2 $m^{-2}s^{-1}$) were detected from all samples of each system from one leaf per replicate with a portable infrared gas analyser LI-COR (LI-6400 XT, Li-COR Inc., Lincoln, NE, USA). The LICOR setting was as follows: 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance levels (I) [31,32], 500 cm^3 min−1 flow rate, 26 °C leaf temperature, and CO_2 concentration 400 μmol (CO_2) mol (air)–1 (controlled by CO_2 cylinder). Each measurement was made with a minimum and maximum wait time of 120 s and 200 s, respectively, matching the infrared gas analysers for 50 μmol (CO_2) mol (air)–1 difference in the CO_2 concentration between the sample and the reference before every change in I. The leaf-to-air vapor pressure difference (VPD) was set to 1.5 kPa, continuously monitored around the leaf during measurements, and maintained at a constant level by manipulating the humidity of incoming air as needed. The measurements have been made during the period of 8:30–11:30 a.m.

Furthermore, the plants were harvested by cutting them at the collar level and were immediately weighed to detect the fresh weight (FW, g). In addition, samples from each cultivation system were transferred to oven at 70 °C for 48 h to measure dry weight (DW). Next, the dry matter content percentage (DMC%) was calculated using the following formula [33]:

$$\boldsymbol{DMC}(\%) = \frac{\boldsymbol{Dry\ weight}}{\boldsymbol{Fresh\ weight}} \times 100$$

2.4. Determination of Chlorophyll and Carotenoid Content in Lettuce Leaves

For the determination of pigment concentration, the method reported by Farahanian et al. [34] was applied. Briefly, 500 mg of homogenised lettuce sample was thoroughly mixed with 10 mL acetone/water solution (80%) in a centrifuge tube. Afterwards, the mixture was centrifuged at 8000× g for 10 min. The extraction was repeated with additional extraction solution to fully extract the pigments. Acetone/water mixture was used to adjust the total volume to 25 mL. The pigment concentration was spectrophotometrically measured at 440, 645, and 663 nm. Chlorophyll a (Chl a), Chlorophyll b (Chl b), and carotenoids were calculated as follows:

$$\text{Chlorophyll a (mg g}^{-1}\text{): } (12.70 \times A_{663} - 2.69 \times A_{645}) \times V/(1000 \times W)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{): } (22.88 \times A_{645} - 4.67 \times A_{663}) \times V/(1000 \times W)$$

$$\text{Carotenoids (mg g}^{-1}\text{): } (470 \times A_{440} - 2.17 \times A_{663} - 5.45 \times A_{645}) \times V/(1000 \times W)$$

where A is the absorbance, V is the volume of the extract (25 mL), and W is the weight sample (0.5 g).

2.5. Antioxidant Compound Extraction

Antioxidant extraction was carried out using the method applied on red lettuce by Flores et al. [35] with some modifications. Lettuce samples were frozen at −80 °C, lyophilised (VirTis lyophiliser—SPscientific, Gardiner, NY, USA) and reduced to powder. Ten mL of methanol/water solution (70:30) was mixed with 0.5 g of lyophilised lettuce, stirred for 60 s, and placed in a Sonoplus Ultrasonic homogeniser, Series 2000.2, HD 2200.2 (BANDELIN, Ultraschall seit 1955, Berlin, Germany), for 10 min at 20 °C and 59 khz. Then, samples were centrifuged at 7000× *g* for 8 min at 4 °C in a refrigerated centrifuge (Sigma 3-16KL, Osterode am Harz, Germany); the supernatant was filtered with syringe filters (PTFE 0.45 μm) and collected until phenol content, and antioxidant activity analysis was conducted. For each sample, four extracts were prepared.

2.6. Phenol Content (PC), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), and 2,20-Azino-Bis Acid (3-Ethylbenzothiazolin-6-Sulfonic Acid (ABTS) Assays

The phenol content (PC) was evaluated spectrophotometrically following the method reported by Galieni et al. [36] with slight modifications. An amount of 150 μL suitably diluted was added to 500 μL of Folin–Ciocalteu reagent. After 3 min, 1500 μL of Na_2CO_3 (25%) was added and rinsed with 10 mL of distilled water. The solution was kept in dark conditions and at room temperature for 1 h. The PC was determined using a single-beam Agilent 8453 diode-array UV–Visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at 765 nm. Gallic acid standard was used to calibrate the method, and the results were expressed as mg GAE g^{-1} dw. In vitro antioxidant capacity with DPPH and ABTS were evaluated as reported by De Bruno et al. [37]. The percentage of inhibition achieved by decolourisation of the reaction solution due to radical extinction was compared with a Trolox calibration curve. The results of both methods were expressed as μM Trolox g^{-1} dry weight of lettuce (μM TE g^{-1} dw).

2.7. Greenhouse Covering Materials and Measurements

On the outside of the glass, the greenhouse was equipped with opaque white photoluminescent bands. These bands consisted of rare-earth light photo-conversion pigments such as Europium red pigment (Re_2O_2S: Eu) and Dysprosium blue pigment ($Sr_2MgSi_2O_7$: Eu, Dy), whose function is to filter sunlight or artificial light, preferably white, to re-emit frequencies between 400 and 700 nm. The latter are close to the peaks of chlorophyll photosynthesis. These bands are under patent by the company SERRANOVA S.R.L. (Perugia, Italy) whose invention was made by Stefano Chiocchini, so the exact composition and production process have not been made public [20]. The photoluminescent bands evenly covered the surface of the greenhouse, so the lettuce parameters were not affected for all cultivation methods.

Other measurements were obtained on a sunny day for 12 h from 07:00 a.m. to 19:00 p.m., including e-PAR and temperature (°C) with an SM-500 Guardian Multi-Sensor

Monitor (Apogee Instruments, Inc., North Logan, UT, USA) and solar radiation with a pyranometer (LSI LASTEM SRL, Milan, Italy) (31 May 2024) for greenhouse and open field. The latter were taken in both indoor and outdoor settings.

2.8. Statistical Analysis

One-way ANOVA (Analysis of Variance) was conducted by SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA) to assess the variance between the cultivation methods and Tukey post hoc test was applied at $p \leq 0.05$ for mean comparisons. Data were reported as mean value ± standard error. Then, to reduce and better visualise the dataset considered for describing the existing relationships between the analysed parameters and cultivation methods, principal component analysis (PCA) was performed by Lê et al. [38] package in R environment.

3. Results

3.1. Morphological and Physiological Parameters

Lettuce growth varied significantly with the cultivation method. The aeroponic system (A) produced the highest fresh weight (FW) at 52.70 ± 5.89 g, while the HSO system yielded the lowest at 11.19 ± 1.00 g. PSI and HSN showed no significant difference between them (Table 1). The NLT was higher in HSN compared to all other systems, with higher numbers of leaves produced. This result is corroborated by Majid et al. [4], who compared a hydroponic deep-water culture system with a soil-based in a polyhouse. The highest number of leaves was recorded in the hydroponic system. Thus, between PSO and HSO and between PSI and A, respectively, there were no statistically significant differences (Table 1). The lower DMC% resulted in PSI than other systems. PSO showed the lowest fresh weight (FW) compared to system A and the other systems, except for HSO. However, its dry matter content (DMC%) was comparable to that of A (Table 1). Among the hydroponic systems, HSN achieved a higher FW than HSO, which exhibited the lowest value.

In this study, it is seen that the trend of plant height growth from transplanting to harvesting is different for the various cropping systems, and in this case, PSI results in greater height over time, followed by A, HSN, PSO, and HSO (Figure 1).

Table 1. Average Tukey post hoc test of different morphological parameters for aeroponics, pot system outdoor (PSO), pot system indoor (PSI), hydroponic nutrient solution (HSN), and hydroponic organic solution (HSN). The means are reported as ± standard error.

Cultivation System	Plants Height (cm)	Fresh Weight (g)	Dry Weight(g)	Dry Matter Content (%)	Number True Leaves	Leaf Width (cm)
A	19.20 ± 1.00 ^{ab}	52.70 ± 5.89 ^{a}	3.19 ± 1.08	7.14 ± 1.88 ^{a}	14.3 ± 0.54 ^{b}	11.35 ± 0.40 ^{a}
PSO	9.30 ± 0.37 ^{c}	25.24 ± 1.29 ^{c}	1.88 ± 0.09	7.54 ± 0.31 ^{a}	10.1 ± 0.35 ^{c}	7.90 ± 0.26 ^{b}
PSI	20.90 ± 0.53 ^{a}	40.28 ± 1.78 ^{b}	1.42 ± 0.19	3.28 ± 0.51 ^{b}	14.6 ± 0.28 ^{b}	10.42 ± 0.20 ^{a}
HSN	17.00 ± 0.39 ^{b}	36.78 ± 2.59 ^{b}	3.01 ± 0.54	8.53 ± 0.17 ^{a}	16.8 ± 0.738 ^{a}	10.17 ± 0.44 ^{a}
HSO	8.17 ± 0.42 ^{c}	11.19 ± 1.00 ^{d}	0.93 ± 0.10	9.02 ± 0.36 ^{a}	11.2 ± 0.16 ^{c}	6.58 ± 0.38 ^{b}
Sign.	***	***	NS	*	*	**

Ns, *, **, *** indicate non-significant and significant at $p \leq 0.01$, 0.05, and 0.001, respectively. The same letters within a column represent not statistically significant differences

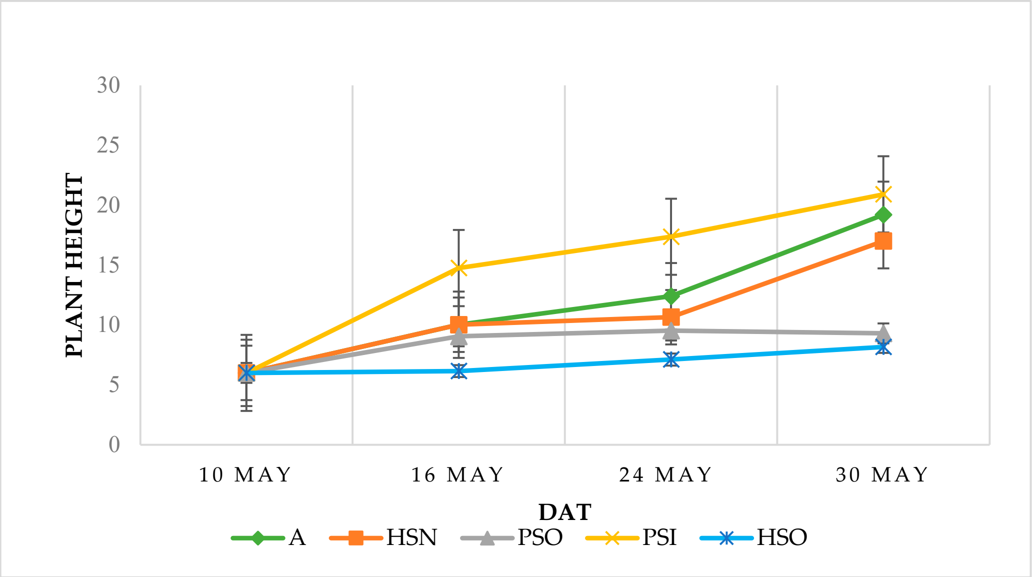

Figure 1. The time course of the plant height in different soilless systems and PSO during the cultivation period from 10 to 31 May.

Leaf width (Lw) did not differ significantly among the A, PSI, and HSN systems; however, it was significantly greater compared to the PSO and HSO systems (Table 1).

The HSO system pointed out a higher and statistically significant photosynthetic rate (18.83 ± 2.17 μmol CO_2 $m^{-2}s^{-1}$) with respect to the PSO and PSI systems and water use efficiency (7.00 ± 0.44 μmol CO_2 μmol^{-1} H_2O) with respect to PSI (Table 2). However, the PhsR and WUE of the HSO were higher than HSN (+12.08% and +14.4%, respectively) and A systems (+19.2% and +11.5%, respectively) but not statistically significant (Table 2). Differently to the PhsR and WUE, the transpiration rate (TrspRa) and stomatal conductance (StoCon) have been not modified by the different cultivation systems (Table 2).

Table 2. The physiological parameters of the different growth systems. The values indicate means ± standard error (ANOVA, Tukey's test at $p < 0.05$).

	WUE ***($\mu mol\ CO_2\ \mu mol^{-1}\ H_2O$)***	**Transpiration Rate** ***($\mu mol\ H_2O\ m^{-2}s^{-1}$)***	**Stomatal Conductance** ***($mmol\ H_2O\ m^{-2}s^{-1}$)***	**Photosynthetic Rate** ***($\mu mol\ CO_2\ m^{-2}s^{-1}$)***
A	6.28 ± 0.36 ^{ab}	2.51 ± 0.30 ^{a}	0.12 ± 0.02 ^{a}	15.80 ± 1.77 ^{ab}
PSI	5.10 ± 0.36 ^{b}	2.01 ± 0.30 ^{a}	0.09 ± 0.02 ^{a}	10.01 ± 1.77 ^{b}
PSO	5.76 ± 0.36 ^{ab}	1.80 ± 0.30 ^{a}	0.08 ± 0.02 ^{a}	10.19 ± 1.77 ^{b}
HSN	6.12 ± 0.36 ^{ab}	2,74 ± 0.30 ^{a}	0.13 ± 0.02 ^{a}	16.80 ± 1.77 ^{ab}
HSO	7.00 ± 0.44 ^{a}	2.75 ± 0.37 ^{a}	0.14 ± 0.02 ^{a}	18.83 ± 2.17 ^{a}
Sign.	** $p < 0.05$	**NS**	**NS**	**

Ns, ** indicate non-significant and significant at $p \leq 0.05$. The same letters within a column represent not statistically significant differences

3.2. Chlorophyll, Carotenoid Content, and Antioxidant Activity

The statistical analysis revealed no significant differences in chlorophyll a (Chl a) and chlorophyll b (Chl b) content between the A and PSI systems.

However, the latter exhibit significant differences for Chl a and b with the HSN and HSO systems, with no discernible distinction between these two. The lower amount of Chl a and b was found in the PSO cultivation system, which was different to other systems.

Carotenoids (Carot), on the other hand, showed a higher value in the A system. The PSO, HSN, and HSO systems showed no differences between them but were significantly different from the A and PSI systems. The last two systems are not statistically different from each other; therefore, the highest Carot content ranged from 0.18 ± 0.04 to 0.1 ± 0.02 for A and PSI systems, respectively (Table 3).

The total polyphenol content (TPC) showed that the PSO system (193.5±20.87 GAE mg g-1 DW) was significantly higher, while the A system (29.33 ± 5.7 GAE mg g-1 DW) had the lowest value. No significant differences were found between the PSI, HSO, and HSN systems, which were significantly different from the A and PSO systems. To evaluate the antioxidant activity of the differently grown lettuce cultivation systems, two assays (DPPH and ABTS) were used. The 2,2′-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid) (ABTS) and diphenylpicrylhydrazyl (DPPH) assays evaluate the ability of polyphenolic extracts from foodstuffs such as lettuce to act as scavengers against these two radicals [39]. The antioxidant activity determined by DPPH was found to have the highest value of 1230.84 ± 124.32 (mm Trolox g-1 dw) and the lowest value of 71.25 ± 14.09 (mm Trolox g-1 dw) for PSO and A systems, respectively (Table 3). PSI and HSN were not statistically different from each other but instead showed significant differences from A, PSO, and HSO. An intermediate value was found for HSO (124.85 ± 24.16 mm Trolox g-1 dw).

Table 3. Chl *a* and *b*, carotenoids, total phenolic content, DPPH, and ABTS mean standard error values from Tukey's test.

(mg g^{-1})	*Chl a*	*Chl b*	*Carotenoids*	*TPC (GAE mg g^{-1} DW)*	*DPPH (mm Trolox g^{-1} dw)*	*ABTS (mm Trolox g^{-1} dw)*
A	0.75 ± 0.03 ^{a}	0.29 ± 0.01 ^{a}	0.18 ± 0.02 ^{a}	29.33 ± 1.34 ^{c}	71.25 ± 3.64 ^{c}	121.24 ± 7.32 ^{d}
PSI	0.67 ± 0.03 ^{a}	0.28 ± 0.02 ^{a}	0.1 ± 0.01 ^{b}	58.75 ± 3.39 ^{b}	189.11 ± 13.39 ^{b}	427.17 ± 18.70 ^{b}
PSO	0.27 ± 0.03 ^{c}	0.09 ± 0.01 ^{c}	0.14 ± 0.01 ^{ab}	193.5 ± 6.02 ^{a}	1230.84 ± 39.31 ^{a}	1113.34 ± 26.85 ^{a}
HSN	0.48 ± 0.04 ^{b}	0.19 ± 0.02 ^{b}	0.13 ± 0.01 ^{ab}	54.14 ± 3.37 ^{b}	213.13 ± 12.64 ^{b}	216.2 ± 14.14 ^{c}
HSO	0.61 ± 0.06 ^{b}	0.17 ± 0.02 ^{b}	0.13 ± 0.01 ^{ab}	60.62 ± 5.28 ^{b}	124.85 ± 12.08 ^{bc}	261.67 ± 34.74 ^{c}
Sign.	**	**	**	**	**	**

The double ** indicate $p < 0.05$. The same letters within a column represent not statistically significant differences

The ABTS assay showed the highest value for PSO (1113.34 ± 93.02 mm Trolox g-1 dw), followed by PSI, HSO, and HSN and the smallest for A (121.24 ± 24.28 mm Trolox g-1 dw). The cultivation systems are all statistically different from each other, excluding HSO and HSN, whose values are not significant between them (Table 3). The antioxidant activity is associated with a high level of phenolic compounds [40], and the PSO system resulted in higher content and radical scavenging activity.

3.3. Total Irradiance, e-PAR, and Temperature

The e-PAR is measured in e-PPFD (extended photosynthetic photon flux density) µmol/m^2/s and solar radiation in W/m^2. In Figure 2, at each hour, PPFD in the e-PAR region showed different values in the indoor versus outdoor environment, with maximum values of 2300 µmol/m^2/s and 1850 µmol/m^2/s for the outdoor and indoor environments, respectively. There were also, for the indoor environment, a few e-PPFD dips during the day with values around 500 µmol/m^2/s (Figure 2). Similarly, solar radiation during the same hours showed maximum values of 736 and 406 W/m^2 for the outdoor and indoor environment, respectively. During the day, there were differences in temperature between the greenhouse environment and the open field (Figure 3). Additionally, we reported the temperatures recorded during the growth period from 10 to 31 May 2024, spanning a total of 21 days. The corresponding graph is presented in the Supplementary Material (Figure S3). In the outdoor environment, the maximum average air temperature throughout the growth period was 24 °C, whereas in the indoor greenhouse environment, it reached 37 °C.

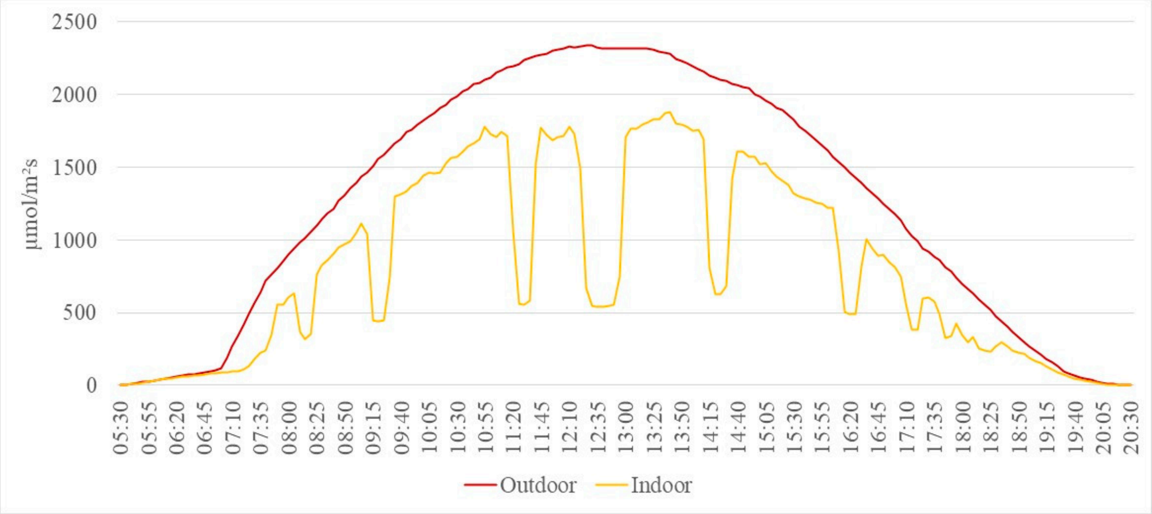

Figure 2. Hourly e-PAR values for the outdoor environment and greenhouse interior.

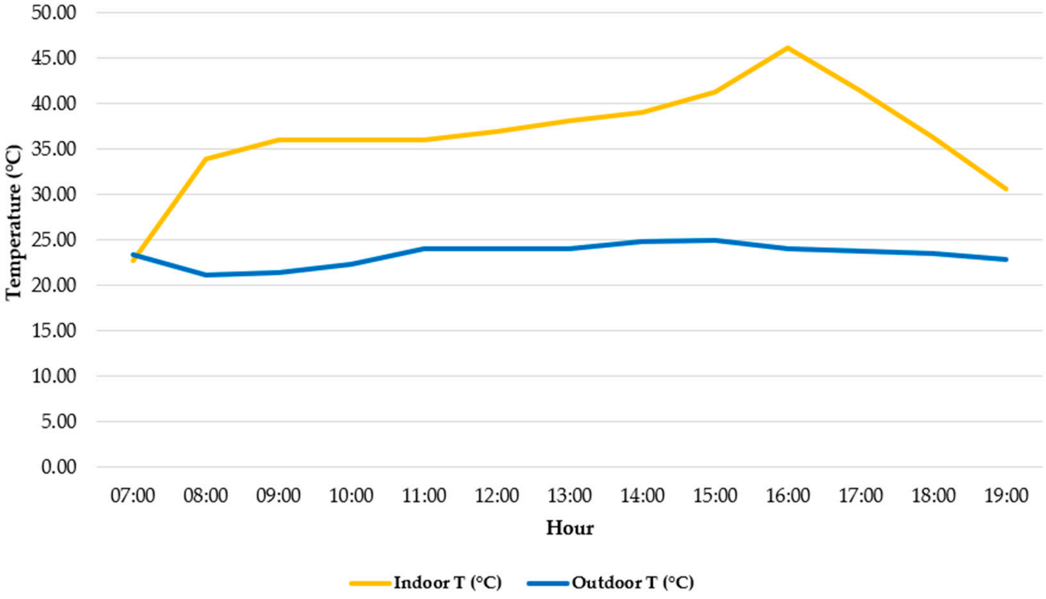

Figure 3. Hourly temperature values for the indoor and outdoor environment during the harvest day (31 May).

3.4. PCA Analysis

Further, the data were subjected to a principal component analysis (PCA) with the aim of studying the relationships between the original variables to find a new, smaller dataset to express what is in common with the original data. This allowed the identification of common factors while maintaining explained variability. PCA is a methodology that is performed when the pattern of correlation between items is high. The investigation offered a comprehensive perspective on the impact of various growing systems on the primary morphological, physiological, and qualitative parameters compared to the control culture of lettuce in outdoor pots (PSO) and indoor (PSI).

To explore the relationships among morphophysiological and biochemical traits across different systems, a principal component analysis (PCA) was conducted (Figure 4). The biplot displays the first two principal components (Dim1 and Dim2), which together account for 67.2% of the total variance (Dim1 = 42%, Dim2 = 25.2%) (Supplementary Table S1). The first principal component (Dim1) primarily distinguishes the PSO and A systems from the others and is heavily influenced by high loadings of total phenolic content (TPC), ABTS, and DPPH antioxidant activity, all of which are strongly oriented in the negative direction of Dim1.

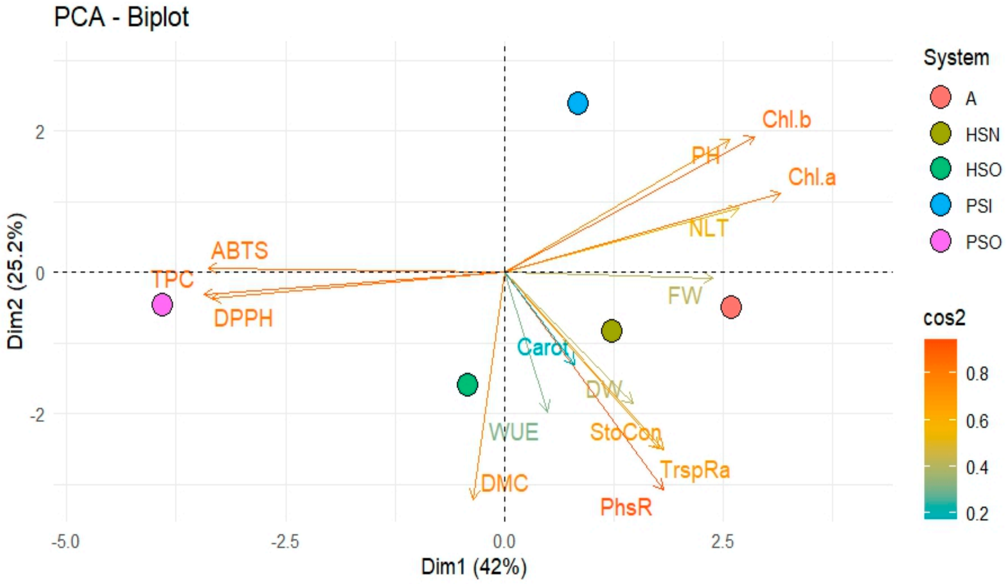

Figure 4. The biplot of PCA.

In contrast, systems such as PSI and HSO are positively associated with variables like chlorophyll a (Chl.a), chlorophyll b (Chl.b), plant height (PH), and the number of leaves (NLT), which contribute substantially to the positive side of Dim1. The second principal component (Dim2) appears to capture variation related to physiological efficiency, with variables such as water use efficiency (WUE), dry matter content (DMC), and stomatal conductance (StoCon) contributing prominently. These traits are oriented more towards the negative direction of Dim2 and are associated with the systems HSN and HSO. Vector length and colour intensity ($\cos^2$ values) indicate the quality of representation of each variable on the factor map. The antioxidant traits (TPC, ABTS, DPPH) and chlorophyll parameters (Chl.a, Chl.b) show strong contributions ($\cos^2 > 0.8$), suggesting that these are key discriminators among the systems analysed (Figure 4). Overall, the PCA highlights distinct clustering of systems, with PSO exhibiting a unique profile characterised by elevated antioxidant traits, while PSI and HSO are more closely associated with photosynthetic pigments and plant growth metrics. This multivariate analysis provides insight into the integrative performance of the systems under study, supporting their differential physiological and biochemical adaptations.

4. Discussion

The results showed that of all the cultivation systems, the aeroponic system had the best production performance in terms of fresh weight, dry matter content, leaf width, the amount of photosynthetic pigments (Chl a and b), and carotenoid content. The relationships between photosynthetic pigments and yield, are important maturity factors [39]. Indeed, parameters such as chlorophyll and carotenoids that are directly related play an important role with crop development, growth, and yield [40].

Lucero et al. [41] demonstrated that in an automated aeroponic cultivation system for a green leaf lettuce culture, leaf growth was more accelerated in the aeroponic culture, with 57% more in the number of leaves, while the width of the leaves was 42% greater than in a traditional system. WenYa et al. [42] carried out a comparison between the biomass production and nutritional quality of lettuce between hydroponic and aeroponic systems under different nitrogen levels. The findings showed that under the same nitrogen level, higher biomass was observed for lettuce grown in aeroponics than in hydroponics. El-Helaly and Darwish [43], cultivating lettuce in hydroponics, aeroponics, and a sandy substrate in a greenhouse, demonstrated that hydroponics and aeroponics had reached 2.51 and to 2.30 fold the yield of the sandy substrate, respectively. The production of the HSO system was inferior to that of the hydroponic system utilising inorganic nutrient solution (HSN) as well as exhibiting reduced antioxidant capability.

Chowdhury et al. [44] evaluated the lettuce production using a liquid organic fertiliser under different hydroponic systems: nutrient film technique (NFT) and deep water culture (DWC) (liquid culture systems) and Dutch bucket (DB) and regular plastic container (RPC) (substrate-based systems). The findings have shown that the growth in RPC was higher than the NFT and DWC systems, respectively, for the shoot width, number of leaves, leaf area, and shoot fresh weight and dry weight. Furthermore, the results of [44] demonstrated that the tested liquid organic fertiliser was better suited for substrate-based hydroponic systems than liquid systems, which supports our findings about the different yields between HSO and HSN. In contrast, in our experiment, the fresh weight of the PSO system was higher than HSO, in accordance with [45], which found that hydroponically grown lettuce fresh and dry weight were lower than conventional soil-based. In this paper, the dry weight in the various systems showed no significant differences (Table 1). Thus, soilless systems have shown higher production than conventional systems. Tunio et al. [11] evaluated the growth and quality of head lettuce in different growing systems, such as an aeroponic system, a hydroponic system, and a conventional growing system. Fresh and dry shoot mass of head lettuce was significantly higher ($p < 0.05$) in aeroponic systems than in hydroponic systems and conventional farming. Ali et al. [46] compared the aeration of a hydroponic system with the aeration of an aeroponic system. The fresh and dry mass of shoots increased in the aeroponic system over those of the hydroponic system. At the physiological level, the soilless systems (A, HSO, and HSN) showed a higher photosynthetic rate and WUE than conventional systems (PSI and PSO) (Table 2). The results are in agreement with those by Thomas et al. [47], in which plants in soilless systems were found to have a higher photosynthetic rate than those in soil, and those in [4], which observed higher WUE and photosynthetic rates in soilless systems than conventional ones. The positive correlation between the photosynthetic rate and plant productivity as observed in lettuce [48] but also in maize [49] is well known. However, the present study contrasted with previous results: no correlation between the photosynthetic rate and fresh weight (Tables 1 and 2). Because the relationship between photosynthesis and productivity is not always straightforward and can be altered by a variety of factors, such as nutrient availability [50], we could assume that the HSO system's high photosynthetic and WUE rate is accompanied by a low yield. This could be owing to poor nutrient uptake by the plant roots as well as differences in root biomass among the systems (Supplementary Figure S2). In fact, as mentioned by [44], organic fertiliser is more suitable for soilless growing systems on solid (perlite, vermiculite, etc.) than in liquid substrates. The amount of chlorophylls and carotenoids was higher in aeroponics than in the PSO system, showing that photosynthetic activity was more efficient. Chlorophyll synthesis requires light, and the concentration also depends on the light spectrum [51]. Snowden and Bugbee [52] found that chlorophyll increases with the light spectrum of blue for several plants but not for lettuce. Aeroponics, on the other hand, was found to have the lowest levels of TPC and antioxidant power. Polyphenols are abundant in plants and constitute a class of aromatic compounds, which have reported many benefits, including antioxidative, anticancer, anti-inflammatory, and visual enhancement properties [53]. The control system PSO had the highest TPC, DPPH, and ABTS contents, followed by PSI, HSN and HSO, and A, most likely because of the higher light exposure compared to all other indoor systems. The latter, in fact, were raised in the open-field PSO system and the greenhouse PSI. Sutulienė et al. [54], however, demonstrated that the growth and quality of lettuce grown in a greenhouse under three different light intensity conditions in spring, with natural light and with LEDs at 150 and 250 μmol/m ^{2}/s, respectively, showed different behaviour for the yield and activity of ABTS, DPPH, and TPC. Controlled illumination boosted new leaf production by 36.8–63.2% (150 μmol/m^2/s) and up to 84.2% (250 μmol/m^2/s) compared to lettuce cultivated under natural light.

ABTS and DPPH were lower in lettuce grown under natural light than in greenhouse lettuce with the two different concentrations. In our case, the trend was opposite, and with an increase in antioxidant activity (Table 3), there was no corresponding increase in the photosynthetic rate (Table 2), chlorophylls, and fresh weight in the PSO system (Table 1). This could be explained by high light exposure and high temperature in spring. In our case, the temperature inside the greenhouse was higher than outdoor during the growth period (37 °C indoor and 24 °C outdoor, respectively) (Supplementary Figure S3), reducing the amount of ABTS, DPPH, and TPC in the other cultivation systems compared to PSO. Furthermore, the high open-field e-PPFD also caused a lower yield in PSO.

In fact, high temperatures are a limiting factor for lettuce production during spring. When lettuce is subjected to high temperatures and high light exposure, it may result in lower yields due to the plants' higher investment in secondary metabolites to defend themselves against abiotic stress [55]. Choi et al. [56] compared lettuce grown in indoor hydroponics with soil conventional agriculture to evaluate ABTS and DPPH radical scavenging activity assays and carotenoid content. In both systems, carotenoid content was found to be the most abundant compound. The polyphenol content and ABTS were higher in conventional soil-grown lettuce than hydroponics. The latter showed high individual phenolic content and DPPH radical activity, corroborating our results. Ahmed et al. [57] investigated the effect of using, in a hydroponic system, an organic nutrient solution (OS) derived from fish waste on the vegetative growth and production of lettuce compared to conventional inorganic hydroponics. The findings revealed that inorganic hydroponics produced the best growth and fresh biomass, whereas the organic nutrient solution produced the lowest results. It was discovered that PSI and HSN had intermediate levels of fresh weight, polyphenols, and antioxidant content. Furthermore, the PSI and A systems showed intermediate values for WUE and the photosynthetic rate, while the organic hydroponic system revealed the highest values for these two parameters. Nevertheless, as previously noted by [44,57], organic hydroponic systems are found to have lower production performance when in liquid culture, and the HSO system had the lowest yield. This may probably be due to a more difficult uptake of nutrients in organic form by the roots due to a lack of direct contact with readily available forms.

These research findings concur with our own that aeroponics outperforms hydroponics and potted systems (PSO-PSI) in terms of production performance. The results of using a PCA to analyse the effects of soilless systems on plant production performance showed that aeroponics was significantly influenced by DMC%, with physiological parameters also contributing to FW yield and chlorophyll quantity. Physiological characteristics also affected fresh weight and dry matter content of the HSN system. PSO control, on the other hand, had an impact on DMC%, which was impacted by a low yield but a larger concentration of phenols and antioxidants. The influence of FW was impacted by indoor PSI and HSO.

For the HSO system, the physiological parameters improved, but the fresh weight was not satisfactory, most likely because the nutrients dissolved in the organic solution were not efficiently assimilated (as mentioned above). For the PSI system, the yield was slightly related to parameters such as chlorophyll without much effect due to photosynthesis and quality parameters.

Photoluminescent bands improve the indoor microclimate by adjusting the light intensity and spectrum for photosynthesis [57,58]. Indeed, the bands re-emit the red light wavelengths (600–700 nm) [19,59,60]. Nishimura et al. [61] demonstrated that under a spectrum conversion film (SCF), the fresh weight of lettuce improved more than lettuce without an SCF. Regarding the content of Chl a and b, Pinheiro et al. [62] showed that for lettuce grown in a hydroponic greenhouse exposed to various photo-selective and control film treatments, the increase in chlorophyll was under photo-selective films. These were divided as follows: red photo-selective film (RF), blue photo-selective film (BF), and control (C-no photo-selective film) on Vera cultivars of lettuce. The results showed that the amount of Chl a of cultivar Vera was under the control, blue film, and red film by 0.31, 0.33, and 0.27 mg-g FW, respectively. The Pinheiro [62] study suggests that hydroponic-grown lettuce shows a better chlorophyll performance content when cultivated under photo-selective films. Taking into account the importance of the quality and quantity of light for the growth and development of plants, the measurements of light parameters have shown different outcomes. The lighting measurements made inside the greenhouse showed that the e-PAR intensity inside the greenhouse was lower than in the outdoor environment by 19.5%, and at the same time, peak e-PPFD lowering with values of 500 μmol/m^2/s in the indoor environment showed a decrease compared to the outdoor environment of 78% (Figure 2). Similarly, the solar radiation inside the greenhouse had a decrease of 44.8% compared to the outdoor environment. This might suggest that the photoluminescent bands may have a shading effect inside the greenhouse, thus reducing the high light exposure similarly to the study of [58]. Indeed, in this last investigation, the PAR intensity in both the outdoor and indoor environment (with photoluminescent bands) was 2042 and 529 μmol/m^2/s, respectively. However, due to technical problems, the limitations of our study concern the lack of further measurements, such as the spectral quality of light and plant fluorescence. Indeed, these measures could have helped to observe potential effects of photoluminescent bands on plant quality; therefore, no further evaluation is conceivable in this regard. Moreover, this goes beyond our main purpose, which is to compare different greenhouse and open-field cultivation systems. In any case, these two aspects, i.e., the employment of photoluminescent bands and likewise soilless systems that improve quali–quantitative performance, have been widely demonstrated individually in the literature [17,18,24–26,29,40–42,46,62], and when combined, they may have an effect on the yield and quality, as stated by [20]. Thus, the use of photo-selective shading screens or photoluminescent bands as an appropriate agronomic technique to improve lettuce-growing conditions [55].

5. Conclusions

In conclusion, the present study revealed that lettuce plants grown in the greenhouse with the use of photoluminescent bands had the following results: The aeroponic (A), hydroponic with inorganic nutrient solution (HSN), and the indoor pot system (PSI-control) had the best production and quality performance, with the best yields for aeroponics but low amounts of antioxidants and polyphenols. The outdoor pot system (PSO) had a higher amount of polyphenols but a low yield compared to the other three mentioned above.

Finally, the organic hydroponic system showed the worst results in terms of quality and quantity. At the same time, the lighting conditions inside the greenhouse showed a reduction in solar radiation and PPFD compared to the outside, highlighting an improvement in internal light conditions. Indeed, the light plays a crucial role for both the production and quality of plants as well as a potential shading impact from photoluminescent bands. In the future, these outcomes will help us to understand how greenhouse cultivation using soilless methods and photo-selective materials can help increase and improve crop yields and the amount of light inside the greenhouse, addressing issues caused by food shortages in both quality and quantity and avoiding seasonality and weather condition problems. In the Mediterranean region, recent decades have witnessed rising temperatures attributable to climate change, resulting in diminished water availability for agriculture and an increase in extreme weather occurrences [63]. Consequently, enhancing crop yields using technologies like greenhouses and soilless cultivation, which reduce pesticide use and water and fertiliser usage, with innovative cover materials, are promising for the increase in and sustainability of food production.

Organic hydroponics, even if it may be a promising sustainable method to reduce environmental pollution, needs to be deepened in terms of plant interaction, nutrient uptake, and adaptation to growing systems and indoor microclimate.

This study could serve as a foundation for future, more in-depth research into how the combination of photoluminescent bands applied to a greenhouse can affect the productivity and quality of plants grown in different soil-free systems.

The photoluminescent bands are under patent as abovementioned in the text [20].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijpb16030094/s1, Table S1: The different two components of PCA. The values in bold are the significative, and each component is represented by the percentage variability in the middle column. In the right column is the cumulative variance; Figure S1: On the left is the glasshouse; on the right is the indoor equipment; Figure S2: Root average fresh weight of lettuce plants grown with different systems. A root FW (bc); HSN (ab); HSO (bc); PSO (a); PSI (c). Different letters indicate significant differences at $p < 0.05$ (ANOVA, Tukey's test, $n = 6$). The higher root FW yield is represented by PSO, followed by HSN, A, HSO, and PSI systems; Figure S3: The monthly average temperature. As can be seen, the maximum average air indoor T was 37 °C on 12 May, and the maximum outdoor air temperature was 24 °C on 29 May.

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