

RESPONSE to the Reviewer 1.

Dear Reviewer,

We sincerely appreciate your clear, detailed and friendly review. We hope that the work we have done has significantly improved the quality of the presentation of the results in the manuscript. Many of your questions and comments were very useful to us for our future work. If there are any aspects of the revised version that do not meet your expectations, we are willing to continue working with you to improve the manuscript until it meets the high standards expected by MDPI.

Kind regards,

The authors.

To the authors,

1. In the abstract, even if it is said that this research is important because "Creating an optimal light environment is crucial for achieving high yields in controlled environment agriculture (CEA) conditions", I highly recommend to shortly define the novelty and originality of this study. This because the comparison with HPS and LED lamps has been largely investigated for several years up to date, and HPS lamps are poorly chosen compared to LED lamps in indoor cultivation systems and vertical farming, to which the authors focus within the CEA systems.

In the abstract, considering that you studied two very different varieties, a short comment on the effects on this aspect should be added (e.g. presence or lack of variety-dependent effect).

Response 1: We greatly appreciate the reviewer's valuable suggestion. We have adjusted the abstract in response to the comments of our reviewers:

Lines 15-33:

Creating an optimal light environment for different crops is crucial for achieving high yields under controlled environment agriculture conditions. Currently, there are no optimal technologies, including lighting technologies, for growing root crops (in particular radish) in CEA. This study examined the effects of HPS and three original sunlike full-spectrum LED lamps on the morphophysiological characteristics and the diffuse reflectance indices of leaves of two contrast radish cultivars. It was found that a higher blue light content (24%) in the spectrum of the LED 3 lamp contributed to the formation of radish plants with a more compact leaf rosette and maximum yield of roots (up to 19%) compared to the other two types of LED lamps. When treated with LED 3, photosynthesis efficiency was probably higher compared to LED 1 and LED 2, which led to a significant decrease in reflected radiation, especially in the blue and red ranges (by 5-143% and 32-86%, respectively). It was found that the genotype had a significant effect on all morphophysiological parameters of radish, while lightning treatment only affected the integral parameters (Pr – proportion of root crop, and Ai – attraction index) and leaf thickness. However, lightning treatment exhibited a greater impact on leaf reflection indices compared to genotype, especially those related to chlorophyll content. The results of the study indicate that LED 3 lamps, simulating natural light at midday, are suitable for production of radish root crops under CEA

conditions, since the mass of root crops and yield per square meter did not differ from those of under HPS reference treatment.

2. Paragraph 77-88 References should be added.

Response 2: Thank you for your suggestion. Links have been added and the text has been updated:

Lines 77-109:

Optical methods of non-invasive control, which make it possible to evaluate the growth and the health of plants and their response to the applied treatments in the genotype-environment system, can serve as an effective tool for optimizing technologies for growing various crops in CEA. The optical properties of leaves are primarily determined by the photosynthetic pigments in their tissues (chlorophyll a, b, and carotenoids), but also depend on leaf structure, the presence and concentration of non-photosynthetic compounds (anthocyanins, flavonols), and water. These properties are a key characteristic of plants, which makes it possible to estimate the amount of photosynthetically active radiation absorbed, the intensity of photosynthesis and, ultimately, the physiological state, as well as predict crop yields [14-16].

Qualitative and quantitative changes in the pigment content of plant leaves inevitably lead to changes in their optical properties [15-17]. This is the basis for understanding the mechanisms of plant physiological reactions, resistance, and adaptation, which are essential for developing effective crop-growing technologies and improving methods for rapid phenotyping. To interpret spectral measurements, several reflectance indices have been developed. These indices, in contrast to simple differences between two reflection values at different wavelengths, are normalized values that more accurately characterize leaf reflectivity [18-22]. The authors who developed the formulas for normalized reflectance indices tested them on various plant species and compared with the results of bio-chemical assessment of chlorophyll - ChlRI [18], carotenoids - SIPI [19], anthocyanins - ARI [21,22] amount in leaves. Also, the relationship between the photochemical reflectance index – PRI and the epoxidation of pigments of the xanthophyll cycle, the efficiency of light use and the efficiency of photosynthesis were determined [19]. We have previously used these indices both in field studies and in the CEA for phenotyping and evaluating the dynamics of changes in biomass, pigment composition, and yield prediction of wheat and lettuce plants that are deficient in mineral nutrients and water [14-16,23].

In response to many factors, the spectral and optical characteristics of radiation reflected from the leaf surface change even before external symptoms appear on the plant itself [17]. It has been demonstrated that the reflectance indices can be used to evaluate the impact of lighting quality on plant development [24].

3. In the whole text, when referring to a cultivar, usually or the abbreviation "cv." or the single inverted commas are used, not both at the same time.

Response 3: Thank you very much for your suggestion. We have fixed the reference to a cultivar, now it is cv. Pernot (cv. P), or cv. Peterburgskiy fioletovyy (cv. Pf).

4. Figure 1C. Showing the HPS treatment along with the photos of the three LED treatments would complete the representation, especially because HPS is the control.

Response 4: We greatly appreciate the reviewer's valuable suggestion. Unfortunately, we did not take good photos of radishes under the HPS lamps. We have adjusted Figure 1 and divided it into two figures: Figure 1 and Figure 2:



Figure 1. Radish plants used in the experiment. A – cv. Pernot, B – cv. Peterburgskiy fioletovyy.



Figure 2. Radish under different LED lamps. A – LED 1, B – LED 2, C – LED 3.

5. Line 135 for the number of leaves, the rational of considering the leaf in the count could be added. To give an example, leaves longer than X mm were considered.

Response 5: Thank you very much for your suggestion. We added the rational of considering the leaf in the count:

Lines 153-156:

During the harvest, the main morphophysiological parameters were measured: the fresh mass of the root crop (Rm, g) and leaves (Lm, g), the number of leaves (Nl, pcs.); the number of developed leaves with a leaf blade length of more than 8 cm was taken into account when we calculated an area of radish leaves.

6. Line 144-145 Ei index: I understand that you called it "economic efficiency index" because it refers to the mass economic interest of the plant. However, considering the formula referring to biomass, it is basically a harvest index. The reference to "economic" efficiency, it gives the expectation of an economic calculation, referring to money, and not just to the biomass harvest.

Response 6: Thank you very much for your suggestion. We agree with your comment and have made changes:

Based on the data obtained, the following selection indices were calculated, characterizing the yield:

Lines 153-156:

1. Pr – the proportion of root crop:

$$\text{Pr} = \text{Mass of the root} / \text{Mass of the whole plant}$$

7. Line 150. A clarification on the experimental layout is required. Two consecutive experiments were performed? How many repetitions per treatment per experiment? In the current form I understand that you repeated the experiment in the time twice but without replicates. Please, clarify in the manuscript.

Response 7: We greatly appreciate the reviewer's valuable suggestion. We have clarified in the manuscript:

Lines 171-174:

The experiment was repeated twice. In each experiment, the number of plants evaluated was 8; since similar average values of the studied parameters were obtained in both experiments, statistical processing was performed for a combined sample of 16 radish plants.

8. In case you had only two overall replicates of the treatments, this is usually considered not enough to properly support the statistics as normally not less than three replicates are used. Line 162 "(Table 1, Figure 2)". the reference to the table and figure could be moved at line 158 just before the two points.

Response 8: Thank you very much for your suggestion. As we conducted the experiment in controlled conditions with identical environmental factors, we believe this repetition will be sufficient to obtain reliable results. In fact, three experiments were conducted, but we consider the first one to be "tentative"; although the results were similar to the next two, the repetition (number of plants) was lower. We did not use the data from the first experiment when writing the manuscript.

9. **Section 2.2**, A small suggestion on Table 1 and related comments in paragraph 173-184. I see the point of comparing the light spectra of lamps; however, I am not fully convinced of the choice of commenting the statistical differences in the material and methods and in a position where you have not presented yet the statistical analysis section. Maybe it would be better to have the synthetic plots or a schematic description of the lamps and this table somewhere else (results or supplementary material, e.g.). For example, instead of giving only the K of the lamps in lines 160-162, you could add there also the spectral ratios (meaning the values you have in the plots of figure 2), like R:G:B:... X:Y:Z:...

Response 9: Thank you very much for your suggestion. We added the spectral ratios (R:G:B):

Lines 178-188:

After sowing, seedling trays with seeds were placed under specific lighting conditions. Four lighting treatments were used in the study (Table 1, Figure 2). HPS (OOO Re-flax, Moscow, Russia), which we had previously used for breeding of the cv. Peterburgskiy fioletovyi, were used

as a reference. HPS red : green : blue ratio (R:G:B) is 8,5 : 9 : 1 (to ease to count and understand, red is taken as 600-700 nm, green is taken as 500-600 nm and blue is taken as 500-600 nm; and the blue light content is taken as 1).

Three original full-spectrum LED lamps (Figure 2A-C) were developed and manufactured at the Agrophysical research institute (AFI):

- 1) LED 1 with a color temperature of 3000 K, R:G:B is 5,2 : 3,3 : 1;
- 2) LED 2 with a color temperature of 4000 K, R:G:B is 2,3 : 2 : 1;
- 3) LED 3 with a color temperature of 5000 K, R:G:B is 1,5 : 1,5 : 1.

10. Line 195-196, please add when the spectra of reflected radiation from leaf surfaces were recorded, e.g., X days from sowing.

Response 10: Thank you very much for your suggestion. We added information:

Lines 239-244:

The measurements were carried out on the 20th day after sowing, 5 days before harvesting. The diffuse reflectance spectrum of the leaf is displayed on the computer screen as a percentage of the reference source's reflectance and can be saved as a digital file. The resulting leaf reflectance spectra were used to calculate reflectance indices, which are closely related to the activity of the photosynthetic apparatus and the physiological state of plants.

11. Line 220-221. Please add "nm" also to 799.1 and 551.8.

Response 11: Thank you very much for your suggestion. We added nm:

Line 265-266

$R_{NIR} = 799.1 \text{ nm}$, $R_{gr} = 551.8 \text{ nm}$, and $R_{re} = 703.4 \text{ nm}$

12. **Section 2.3.2** there are quite discursive explanations that looks like more appropriate in an introduction to the use and the choice of these indices. Particularly, lines 246-252 about heterogeneity in PRI could fit a discussion section rather than Materials and Methods.

Response 12: Thank you very much for your suggestion. We have partially transferred information about the PRI index to the introduction and removed excessive explanations about heterogeneity in PRI:

Line 97-99

Also, the relationship between the photochemical reflectance index – PRI and the epoxidation of pigments of the xanthophyll cycle, the efficiency of light use and the efficiency of photosynthesis were determined [19].

13. **Section 3.1** suppose it should be called "Results and Discussion" and not just "Results".

Response 13: Thank you very much for your suggestion. In the new version of the manuscript, Section 3 is called "Results and Discussion" (Line 311).

14. Lines 269-273. Please add references to the tables/figures where the reader can check the info. Lines 279-282 repeat the concept, so I would suggest making these lines concise.

Response 14: We greatly appreciate the reviewer's valuable suggestion. We have significantly rewritten the section 3.1, applied a new method of statistical data processing and description, and removed unnecessary repetitions:

Lines 323-325:

Genetically determined differences between the cultivars were generally found in all studied parameters, except for LA (Figures 4A-E, Table S1). Higher root mass in all treatments for cv. Pf can be explained by transgressive breeding [6].

15. Figure 3 caption. It is useful to the reader the explanation in the caption of the abbreviations in the figures. For example: A- root mass (Rm); B - leaves mass (Lm), etc...

Response 15: Thank you very much for your suggestion. The latest version of the manuscript presents a modified (after additional statistical processing) version of this figure, now it has the number 4. We've deciphered all the abbreviations in the caption:

Lines 338-344:

Figure 4. Effects of crop variety (Pernot (P) and Peterburgskiy fioletovyy (Pf)) and spectral lighting treatment (HPS, LED 1, LED 2, LED 3) on growth and biomass parameters. Data are presented as mean $\pm$ standard error. For each subfigure, uppercase letters denote significant differences between cultivars, and lowercase letters denote significant differences among lighting treatments (Tukey's HSD test, $p < 0.05$). (A) Root mass (g). (B) Leaf mass (g). (C) Proportion of root crop (Pr). (D) Attraction index (Ai). (E) Number of leaves. (F) Leaf thickness (μm).

16. About 3. Please, apologize for the long text, is to be as clear as possible. The current representation with letters referring to all the single combinations of light*genotype is misleading to the reader. Checking table S1, the interaction is significant only in Root shape index, so why presenting the posthoc letters of the interaction for all the parameters? To check the significance of main effects and interactions, the reader is obliged to check the p value in supplementary table, which are "supplementary" but essential for the understanding of the results.

Response 16: We greatly appreciate the reviewer's valuable suggestion. We conducted an additional statistical analysis of the data obtained, including post-hoc analysis. The revised version of the manuscript includes all new statistical results, Figure 3 (now Figure 4), as well as tables 3-5, now contain letters that reflect genetic and environmental differences (Different letters in the same reflectivity index indicate significant differences between treatments:

uppercase letters denote significant differences between cultivars, and lowercase letters denote significant differences among lighting treatments (Tukey's HSD test, $p < 0.05$). Table S1 has also been rewritten, in particular, all the characters that are not mentioned in the text of the manuscript have been removed from there. Accordingly, we have rewritten the text of sections 3.1 and 3.2 in accordance with the obtained values.

17. Discussion in lines 373 to 411 could be more concise on the general description of spectrum-plant relation.

Response 17: Thank you very much for your suggestion. The discussion on lines 373-411 (in revised version – on lines 410-411) has become shorter, and all this part of manuscript has been rewritten:

Lines 411-427:

Blue and red light play a key role in photosynthesis, although their effects on plants can be different. Many publications have demonstrated the stimulating effect of red light on the height, diameter, and aboveground biomass of various crops, in particular, lettuce [30-32], pak choi [33], and tomato [34]. In our study, the maximum values of the aboveground part (leaf mass) were also found in the LED 1, which is characterized by the maximum (43%) proportion of red light in the spectrum (Figure 3,4B). Similarly, the presence of blue light is necessary for normal plant growth, and the addition of blue wavelengths in certain proportions (optimal for a specific crop or variety) increases yield and improves quality for a number of crops in CEA [35-38]. At the same time, blue light can also reduce plant growth. High proportions of blue light have been shown to reduce the biomass of the above-ground part of lettuce [39-41], the height and biomass of cucumber shoots [41], and the dry weight of cucumber, pepper, and tomato shoots [42]. Additionally, blue light can stimulate the development of root systems and increase root biomass in various crops [42-45]. Our experiments also showed some inhibition of the growth of the leaves and, simultaneously, stimulation of the growth of the root crop in both radish cultivars under LED 3 treatment, which has the highest proportion of blue light in its spectrum (Figures 3,4A).

18. Lines 458-459 “It was found that the leaves of the cv. ‘Pf’ reflected less PAR radiation and apparently absorbed more”. Where can the reader find this result? If referred to table 3,1 do not see letters of statistic supporting this affirmation.

Response 18: Thank you very much for your suggestion. The letters of statistic supporting were added, and the results were revised:

Lines 479-497:

Significant differences were observed in the amount of radiation reflected from the leaf surface across all spectral ranges, both between the lamps and between the studied cultivars. It was found that the leaves of the cv. Pf reflected less PAR radiation and apparently absorbed more, suggesting more intensive growth and biomass accumulation compared to the cv. P. So, under HPS treatment, the amount of PAR radiation reflected from the leaf surface were 28.8% (cv. Pf) and 40.1% (cv.

P); under LED 1 – 46.1% and 55.2%, respectively; under LED 2 – 37.3% and 43.3%; and under LED 3 – 29.9% and 39.7% for the cvs. Pf and P, respectively.

19. Lines 488-500.1 suggest shortening on this explanation since result and discussion section is already long and this is mainly an explanation on why the authors used the method.

Response 19: Thank you very much for your suggestion. We removed unnecessary information.

20. Lines 504-506. Where can the reader find the results referred to this statement about “patterns of change depending on the PAR spectral composition during the growing season”? I understood that the spectrum was fixed through the growing cycle of the present experiment Data or analysis on variation across the growing season are not reported in results. Please clarify in the manuscript if this sentence refers to a missing citation to another research.

Response 20: Thank you very much for your valuable suggestion. You are absolutely right, this is an incorrect expression, we measured the spectra only once. The expression " during the growing season" has been removed.

21. Lines 506-507, 514-515. “The smallest differences in this indicator were found between the HPS and AFI-3, and the largest between the HPS and AFI-1”. Same as above, the letters of statistical analysis are mainly the shared, indicating no significant differences.

Response 21: Thank you very much for your valuable suggestion. The letters of statistical analysis are added now, and significant differences are indicated.

22. Table 5. Please add in caption or foot notes the full names of the abbreviation used as reflectivity index column.

Response 22: Thank you very much for your suggestion. The caption was added:

Lines 558-564:

Note: Data are presented as mean $\pm$ standard error ($M \pm SEM$). Different letters in the same reflectivity index indicate significant differences between treatments: uppercase letters denote significant differences between cultivars, and lowercase letters denote significant differences among lighting treatments (Tukey's HSD test, $p < 0.05$). SIPI – leaf structure-independent pigment index; ARI-1 and ARI-2 – anthocyanin reflectance indices; PRI - photochemical reflectance index; R800 - light scattering inside leaf tissues. Cv. Pf – cultivar Peterburgskiy fioletovyy, cv. P – cultivar Perno.

23. On the base of the doubtful result interpretation, I kindly suggest reviewing the conclusions, to make them consistent with the results statistically significant.

Response 23: Thank you very much for your valuable suggestion. We have corrected the text in the manuscript in those places where it concerns the reliability of statistical differences, and also corrected the conclusion. We believe that despite the fact that the differences between the treatments in terms of root crop weight and yield were not statistically significant, the trend we identified associated with an increase in these indicators with an increase in the proportion of blue light in the spectrum of the LED lamp is quite reliable. Therefore, we consider it possible to write about this (as a trend), especially since the reflection indices also confirm our conclusions.