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Statistical Optimization of a Hyper Red, Deep Blue, and White LEDs Light Combination for Controlled Basil Horticulture

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Featured Application: The specific application of this work is related to basil cultivation in indoor horticulture under artificial light. This work is devoted to the investigation of specific light combinations, based on hyper red, deep blue, warm white LEDs, to promote basil germination and growth. The aim is to improve basil's yield and quality by reducing the overall growth cycle at the same time.

Abstract: This study aims to optimize artificial LEDs light conditions, for "Genovese" basil germination and growth in an indoor environment suitable for horticulture. Following a previous study on the synergic effect of LEDs light and a tailored fertilizer, in this study, the effect of white LED in combination with hyper red and deep blue, as well the plants–lights distance, was correlated to 14 growth and germination parameters, such as height, number of plants, etc. A design of experiments approach was implemented, aiming to derive mathematical models with predictive power, employing a restrained number of tests. Results demonstrated that for the germination phase, it is not possible to derive reliable mathematical models because almost the same results were found for all the experiments in terms of a fruitful germination. On the contrary, for the growth phase, the statistical analysis indicates that the distance among plants and lights is the most significant parameter. Nevertheless, correlations with LED light type emerged, indicating that white LEDs should be employed only to enhance specific growth parameters (e.g., to reduce water consumption). The tailored models derived in this study can be exploited to further enhance the desired property of interest in the growth of basil in horticulture.

Keywords: basil; design of experiments; LED

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1. Introduction

The principal mechanism that drives plants germination and growth is photosynthesis, as light is the primary source of energy and the principal regulatory variable in plant's cycle. Thereafter, light determines the appearance of plants, their growth rate as well as their quality. In a closed environment, plant growth and morphogenesis can be controlled as desired with artificial light, and, for this reason, it has been demonstrated that the annual production capacity of plants per floor area is about 10 times higher than in the greenhouse [1]. Since under natural light source (that include all the visible spectra), plants must constantly respond with biochemical (carbohydrate content and pigment) and physiological (nutrient uptake and photosynthetic rate) adaptations, with artificial light it is possible to meet plants requirements, therefore improving the overall growth efficiency [2,3]. For example, low irradiance leads to a less controlled leaves growth, breaking the optimization mechanism that regulates the leaf area per unit necessary to maximize the light interception [4] in the growth period of the plants.

In addition, an increased efficiency in plant germination and growth, by a precise control over artificial light, can be a relevant opportunity for environments where natural light is naturally absent, such as northern latitude countries or space travel. Artificial light optimization can be beneficial for leafy greens and herbal crops that are an exceptional source of various human health-promoting macronutrients such as polyphenols, vitamins, and essential oils [5]. For this reason, for these plants, the relationship between artificial light and physiology under different light spectra and/or light intensities is attracting the interest of the more recent research [6,7]. Among leafy greens, *Ocimum basilicum* L. (Basil) belonging to the *Lamiaceae* family is a worldwide-spread herbal crop employed as culinary herb and for essential oil extraction, as well as, widely used in medical industry as component of oral health products and to treat several medical complaints such as gastrointestinal disorders, headaches and stomach aches [8,9]. Considering the beneficial effect on human health and nutrition, basil is considered one of the best candidates to study its potential germination and growth optimization through artificial light [10].

As artificial light source for plant cultivation, light-emitting diodes (LEDs) have received considerable attention as they have the capability to produce a considerable light intensity with low radiant heat output for years, compared to fluorescent lamp, therefore with an increased lifespan [11]. Compared to incandescent light's 1000 h and fluorescent light's 8000 h life span, LEDs have a significantly longer life of 100,000 h [12]. In addition, by using well-tailored LEDs-based arrays, it is possible to have a full control of the spectral composition and an adjustment of light intensity for a specific plant or growth period. In terms of power consumption, the beneficial effect related to the use of LEDs as light source is twofold [13–15]. First, it is well known that, for a given light intensity, the power consumption of a generic LED lamp is significantly lower than any other artificial light source. Second, for a given plant, realizing a custom LED light source accordingly with the optimal light combination of the plant, avoids power waste related to light wavelengths that have no effects on the growth of the plant. These considerations are valid not only for a monochromatic LED, but also for white LEDs. It is well known that white LEDs do not produce directly white light. There are two main manufacturing process to realize white LEDs. The first one is a conventional process that exploits RGB-LEDs dies on the same chip. In this case, it is possible to change the color temperature of the produced white light by acting on the current flowing into the LED that in turn vary the light intensities of the single RGB-LEDs. The second one is called fluorescence (GaN-LED) and exploits a blue LED (or UV in some cases) only with a special yellow coating realized with phosphor. In this case, a phosphor coating is deposited on the blue LED die. The color temperature of the obtained white light depends on the dominant wavelength of the blue LED used [15].

According to the more recent research, plants' chlorophyll molecules absorb the red and blue spectral components most efficiently, and for this reason, red and blue spectral components best drive photosynthetic metabolism and are the most relevant for crop growth [16]. In addition, exposure to red and blue light increases the content of phenolic compounds and improve antioxidant activity in various leafy greens with respect to only white light exposure, including if these effects are different among the species [16–18]. Blue light, having short wavelength, has the capability to regulate plant growth at different stage, promoting, when in considerable presence, compactness, and plant density [19–22]. Red light, having longer wavelength, has beneficial effect on stem elongation and leaf area [23,24]. However, green and yellow light components are less compatible to plant receptor, and they are principally reflected or transmitted and thus are not as important in the photosynthetic process. For basil cultivation the main works are focused on phenolic biosynthesis and accumulation but few of them are related to systematic cause–effect investigation on growth and yield performance under artificial lamps [1,7]. In addition, up until now, few studies investigate this effect in a systematic way and comparison among different studies is not trivial due to the different boundary condition employed for basil cultivation. In particular, hyper red (660 nm) light component effect on plant growth is not fully understood yet, as it seems to contribute to increase the plant' yield and quality, but, at the same time, promoting a reverse effect of phytochromes leading to changes in plant morphology, gene expression, and reproductive responses [25–27].

On these bases, the main objective of the present study is to evaluate the artificial illumination spectrum that is capable to induce the most significant biomass improvement of "Genovese" basil (*Ocimum Basilicum*) plants in a controlled environment. Following the results presented in [28] three different light–plants distances and four different LEDs modules have been considered in combination. From a previous study, the effect of different artificial lights was evaluated as negligible, with respect to a fertilizer addition, to promote basil growth [28]. A slight favorable effect was estimated for a combination of LEDs light containing hyper red and deep blue light in a proportion of 1:3 (LEDs modules quantity), but further investigation was necessary [28]. A statistical approach (design of experiments) has been applied to reduce the number of possible combinations of the variables, and, consequently, of the experiments needed to obtain statistically reliable correlation among data [29]. Data inherent to germination, growth and plant's water consumption have been collected to demonstrate and calculate, numerically, specific effects of artificial light conditions on basil cultivation. Thereafter, as innovative aspects of this study, lights spectral components that have not widely investigated have been studied, and in addition, a systematic approach has been applied, with the aim to calculate specific light combination to further enhance a specific property. Finally, to the best of author's knowledge, this is one of the few researches investigating the effect of different artificial light on plant's water consumption.

2. Materials and Methods

2.1. Materials

For this study, Basil (*Ocimum Basilicum*) seeds belonging to the variety called "Genovese" (Producer: Magnani Sementi) were employed as reference. As burying soil, Floradur B pot coarse universal potting soil (Floragard Vertriebs GmbH, Oldenburg, Germany) was employed. Detailed specifications about this substrate can be found in a previous study, as the same soil was employed to promote the comparison among different soils suitable for basil [28]. This soil respects the limits generally applied to fertilizer compounds necessary to be sold and employed [30].

The experiments were conducted by exploiting as artificial light commercial LED modules realized with OSRAM Oslon®SSL ThinGaN LEDs (UX:3) technology, [31]. The composition in terms of number and type of LEDs are summarized in Table 1. The modules were characterized using an Orb Optronix TEC-100 electrical–thermal–optical (ETO) system with integrating sphere.

Table 1. Composition of the LEDs modules used in the experiments to realize the considered light combinations.

Module Type	Module Code [1]	Total Number of LEDs	Total Number of HR [1] LEDs	Total Number of DB [2] LEDs	Total Number of WW [3] LEDs
1	5HR:1DB:6WW	12	5	1	6
2	9HR:3DB	12	9	3	-
3	6HR:6DB	12	6	6	-
4	3HR:9DB	12	3	6	-

[1] HR = hyper red. [2] DB = deep blue. [3] WW = warm white.

In horticulture, from an application point of view, the main parameter to consider for a light source is the photosynthetic photon flux (*PPF*). The overall photon flux (*PF*) in µmol/s of a light source depends on the radiant flux (*RF*) in W, and is proportional to the light wavelength, λ. Only the portion of photons with wavelengths in the range 400–700 nm (i.e., the so-called photosynthetic active region PAR) contributes to photosynthesis. *PPF* is the portion of PF due to photons within the PAR. Since the ETO system provided the data concerning the whole modules, the contribution of each LED type was calculated off-line. For the hyper red [32] and deep blue [33] LEDs, the PPF contribution of a single LED (i.e., $PPF_{singleLED\_HR}$ and $PPF_{singleLED\_DB}$) were extracted from datasheets using (1) or (2) depending on the specific LED

$$PPF_{singleLED\_HR} \approx PF = RF \cdot \lambda \cdot 0.00836/1000 \quad (1)$$

$$PPF_{singleLED\_DB} \approx PF = RF \cdot \lambda \cdot 0.00836/1000 \quad (2)$$

where, RF is the radiant flux in W, λ is the peak wavelength of the LED in nm, the light wavelength, the coefficient 0.00836 = $1/(h \cdot c \cdot N)$ h is Planck's constant, c is the speed of light and N is the constant of Avogadro. RF and λ are reported in the datasheet.

For a given monochromatic LED, the error derived by approximating PPF with PF is negligible because outside the PAR range there are no photons emitted.

For warm white LEDs, instead, it is not possible to apply directly (1) or (2) due to the intrinsic nature of the white light. Different from single color light sources, white light sources have not a single dominant wavelength in the spectrum. For this reason, the datasheets of white LEDs usually do not report the RF. They report instead the luminous flux (LF, in lumen) for a given color temperature (i.e., 3000 K for the considered LEDs). In this case, to obtain the estimated $PPF_{singleLED\_WW}$ contribution of the warm white LEDs a two-steps empiric procedure was used. First, the relative spectral emission of the considered white LEDs [34], reported in the datasheet was included in the freely available spreadsheet tool provided by lighting analysts [35]. This allowed to obtain a luminous-to-photon flux conversion factor $K_{LF\text{-}to\text{-}PF}$ = 14.64. Second, the obtained conversion factor that was employed in (3) to obtain the PPF of the warm white LED of interest, $PPF_{singleLED\_WW}$.

$$PPF_{singleLED\_WW} = LF \cdot K_{LF\text{-}to\text{-}PF}/1000 \quad (3)$$

The obtained PPF values for each type of LED are summarized in Table 2.

Table 2. Main opto-electrical features of the considered single LED as result by combining data reported in datasheets and off-line computations.

LED Type	Peak Emission Wavelength	Spectral Bandwidth at 50%	Correlated Color Temperature	PPF [μmol/s]
HR	660 nm	25 nm	-	2.01 [1]
DB	451 nm	20 nm	-	2.26 [2]
WW	-	-	3000 K	1.47 [3]

[1] $PPF_{singleLED\_HR}$ obtained by (1). [2] $PPF_{singleLED\_DB}$ obtained by (2). [3] $PPF_{singleLED\_WW}$ obtained by (3).

For each considered LED module, the overall PPF, PPF_{module_calc} can be easily obtained as shown in (4) combining the $PPF_{singleLED\_HR}$, $PPF_{singleLED\_DB}$ and $PPF_{singleLED\_WW}$ reported in Table 2 and the LED's composition of the modules reported in Table 1.

$$PPF_{module\_calc} = \#LED_{HR} \cdot PPF_{singleLED\_HR} + \#LED_{DB} \cdot PPF_{singleLED\_DB} + \#LED_{WW} \cdot PPF_{singleLED\_WW} \quad (4)$$

where $\#LED_{HR}$, $\#LED_{DB}$, $\#LED_{WW}$ are the number of hyper red, deep blue and warm white LEDs per module, respectively.

The obtained PPF compositions for each considered LED module is shown in Table 3. For a given module, there is not a direct relationship between the percentage of PPF and the ratio between number of LEDs of a given type and total number of LED of the module. The reason is that the overall photon flux (PF), in μmol/s, depends on the radiant flux (RF), in W, and is proportional to the light wavelength, λ. Conversely, the energy per photon, E_{ph}, is inversely proportional to λ.

Table 3. Calculation of the PPF composition for each considered module.

Module's Code	PPF HR [µmol/s]	PPF DB [µmol/s]	PPF WW [µmol/s]	Total PPF_{module_calc} [µmol/s]	%PPF HR	%PPF DB	%PPF White
5HR:1DB:6WW	10.07	2.26	8.84	21.17	47.57	10.69	41.74
9HR:3DB	18.13	6.79	-	24.92	72.76	27.24	-
6HR:6DB	12.08	13.57	-	25.65	47.10	52.90	-
3HR:9DB	6.04	20.36	-	26.40	22.88	77.12	-

For each considered module, the comparison between the *PPF* measured with the ETO integrating sphere, PPF_{ETO}, and the PPF_{module_calc}, is shown in Table 4.

Table 4. Comparison between measured and calculated PPF for each considered LED module.

Module's Code	PPF_{ETO} [µmol/s]	PPF_{module_calc} [µmol/s]	Error [1] [%]
5HR:1DB:6WW	19.48	21.17	7.98
9HR:3DB	23.61	24.91	5.23
6HR:6DB	24.63	25.66	4.00
3HR:9DB	24.19	26.40	8.38

[1] Error = 100 * 1 − ($PPF_{ETO} - PPF_{module\_calc}$).

Off-line computations PPF_{module_calc} have a good match with direct measurements PPF_{ETO} with a maximum deviation of about 8%. In the first approximation, this evidence validates the procedures used to calculate $PPF_{singleLED\_HR}$, $PPF_{singleLED\_DB}$ and $PPF_{singleLED\_WW}$, demonstrating that they provide an easy way to estimate the *PPF* of any custom LED light source, basically using only the data provided by the LED manufacturer and always reported in the LED's datasheet. From a practical point of view, this is important because for a given horticulture scenario, it allows to obtain a reliable *PPF* estimation at design time, without need for field tests on the real system. Consequently, it allows obtaining significant savings in terms of development time, costs, instrumentation needed and design software analysis.

To verify the behavior of each LED module under the same working conditions, and to evaluate the effect of the distance among plants and LEDs in terms of PPF, three different distances, d, (60, 70 and 80 cm respectively) were considered. All the considered LED modules were experimental characterized by means of a low-cost spectral sensor (AS7341 from Ams, [36]). The measurements were conducted by reproducing the same test conditions used for the test with ETO integrating sphere, i.e., constant current flowing through the LEDs of 350 mA and temperature of the modules of 42 °C. Temperature and relative humidity of the surrounding air were recorded using wireless sensors with accuracy equal to ±1 °C (resolution: 0.1 °C) for temperature, and ±5% (resolution: 1%) for humidity. The results are summarized in Figure 1. As expected, sensor (i.e., plants)-LEDs module distance affects the effective fraction of PPF. The smaller the distance, the larger the effective PPF, and this is due to the radiation angle of each single LED. While ETO captures each emitted photon, in real conditions, the energy associated with photons that do not reach the plants is wasted. This effect is mitigated thanks to the use of specific lens from LEDiL, [37], embedded into the LED modules, that reduce the radiation angle to ≈30 deg. These test conditions were held fixed for all the experiments described in the following paragraphs.

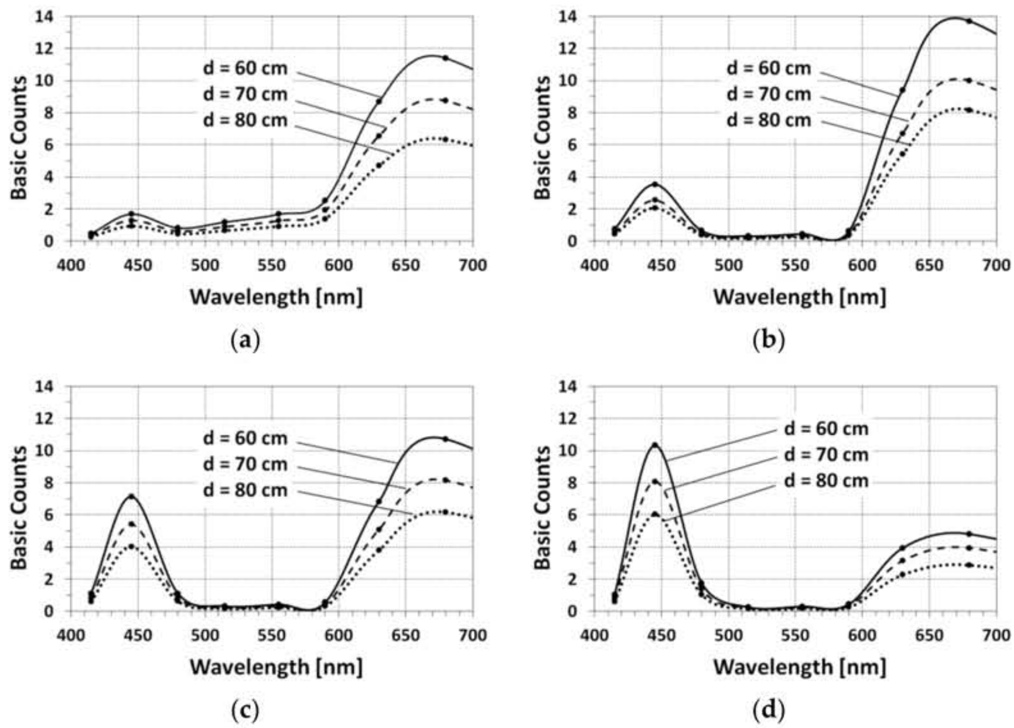


Figure 1. (**a**) Module 5HR:1DB:6WW, (**b**) module 9HR:3DB, (**c**) module 6HR:6DB and (**d**) module 3HR:9DB: light spectrums obtained using the low cost AS7341 spectral sensor. Each curve is obtained by the interpolation of the dimensionless output values of the 8 optical channels of the sensor with filters centered at wavelengths of 415, 445, 480, 515, 555, 590, 630, and 680 nm, in order (marker dots) [37].

2.2. Statistical Methods

A statistical method, known as design of experiments (DoE), was employed to plan the lower possible number of experiments needed to calculate mathematical models, correlating light conditions and germination/growth performances, with predictive power [29]. Three factors were considered as independent variables, as shown in Table 5:

Table 5. Independent variables used for the statistical analysis.

Factor	Type	Levels	Minimum	Zero-Point	Maximum
Distance	Numeric/Discrete	3	60 cm	70 cm	80 cm
HR:DB ratio [1]	Numeric/Discrete	3	1HR:3DB	1HR:1DB	3HR:1DB
White	Categoric/Nominal	2	YES	-	NO

[1] ratio between number of hyper red (HR) and deep blue (DB) LEDs for a given light combination. 1HR:1DB = same number of HR and DB LEDS. 1HR:3DB = number of DB LEDS that is the triple of the number of HR LEDs. 1HR:3DB = number of HR LEDS that is the triple of the number of DB LEDs.

The other variables occurring in the process and not specifically considered in this study, such as humidity and temperature, were kept constant during all the tests, according to the procedure as explained in paragraph 2.3. The Design Expert 13.0 (Stat-Ease) code was used both to plan the experiments and to perform the statistical analysis. To avoid environmental conditioning, all the sample were tested at the same time. Each distance was kept constant for each sample by lifting the light placement by hand, according to plant's increasing high, during growth, that was measured during the experiment with a meter stick having resolution 1 mm.

Due to the high number of possible combinations among factors, a fractional response surface design was selected with I-optimal design type to enhance a lower average prediction variance. A total of 20 experiments were performed, including repetitions of some of them for variance estimation inside a homogenous group of samples, due to intrinsic experimental approximation, with the aim to compare it with the variance among all investigated samples (Table 6).

Table 6. Experimental plan.

	DoE VARIABLES			LIGHT COMBINATION [4]			
#Test	*d* [1] [cm]	HR:DB Ratio [2]	White LEDs	COMBINATION'S CODE [3]	# of HR LEDs	# of DB LEDs	# of WW LEDs
1	80	1:03	YES	LR14	8	10	6
2	80	1:03	YES	LR14	8	10	6
3	80	1:01	YES	LR13	11	7	6
4	80	3:01	YES	LR12	14	4	6
5	80	1:03	NO	LR44	6	18	0
6	80	1:01	NO	LR33	12	12	0
7	80	3:01	NO	LR22	18	6	0
8	70	1:03	YES	LR14	8	10	6
9	70	1:01	YES	LR13	11	7	6
10	70	3:01	YES	LR12	14	4	6
11	70	1:03	NO	LR44	6	18	0
12	70	1:01	NO	LR33	12	12	0
13	70	1:01	NO	LR33	12	12	0
14	70	3:01	NO	LR22	18	6	0
15	60	1:03	YES	LR14	8	10	6
16	60	1:01	YES	LR13	11	7	6
17	60	3:01	YES	LR12	14	4	6
18	60	1:03	NO	LR44	6	18	0
19	60	1:01	NO	LR33	12	12	0
20	60	3:01	NO	LR22	18	6	0

[1] LEDs to plants distance. [2] Number of LEDs ratio between hyper red (HR) and deep blue (DB) LEDs. [3] Code obtained by LEDs module's type reported in the first column of Table 1. e.g., LR14 means light combination obtained by combining a type #1 module (5HR:1DB:6WW) with a type #4 module (3HR:9DB). [4] all the light combinations exploit 24 LEDs as result of different combination of 2 LEDs module chosen among the ones reported in Table 3.

Analysis of variance (ANOVA) was employed to investigate single and synergic effects of the artificial light conditions on basil germination and growth. To apply this type of approach, input variables must be independent of each other and normally distributed in the chosen range. In these conditions, each response variation can be divided into different components to evaluate the effect of each factor, their interactions, and experimental error (or unexplained residual) [29]. F-test was applied to estimate if the variation among all the samples, usually due to process difference or factor changes, is larger enough or not than the variation within samples, thereafter, obtained in same experimental conditions. The p value parameter was employed to evaluate the significance of each factor, in single or in interaction, and of the overall model, as it represents the probability that the considered model or factor is significant (p value < 0.05) or not [38]. R^2 and Pred-R^2 parameters were employed to estimate the quality of the fit for the measured dataset, in terms of regression analysis and predictive power of the model, respectively. R^2 is the proportion of the variance in the dependent variables that is predictable from the independent variables and Pred-R^2 is analogous but associated with predicted values [39]. To better highlight the role of the main components on the final considered properties, response contour plots and mathematical equation were derived and discussed. Finally, the desirability function (D) was employed to balance the different responses, considering their peculiar importance (from 1 to 5, where 5 is equal to the highest importance), and objective with respect to the overall purpose of the work.

2.3. Experimental Methods

Five basil seeds per unit were buried in Floradur B pot coarse universal potting soil in plastic containers having a volume of 450 cm^3 each, with 3 repetitions of the 20 pots described in Table 6, for a total of 60 units. During the test, the position of each pot was exchanged with another of the same sample, to avoid any possible slight difference among repetition due to different area illumination under the LEDs light. Trials lasted 35 days, at the end of which the growth and germination data were collected. The experiments were conducted in a room totally isolated from external light sources. Photoperiod was set to 15 h/day (from 00.00 to 15.00) for 30 days. Temperature was kept almost constant near 19 °C, while humidity was always near 60%, with a variation around 5%. These conditions were maintained for all the trials. Artificial light treatments were applied using a growth structure (Figure 2) divided into different areas. Each area was separated from external sources of light through fixed wood panels as walls. As mentioned before, plants were placed on the bottom in a fixed position at three different distances, *d*, from LED module's position: 60, 70, and 80 cm. The three distances were chosen to ensure a homogeneous illumination respectively in areas equal to 31 × 31, 37 × 37, and 43 × 43 cm. The proportion between these three areas is respectively 1:1.5:2. The fixed distance remained constant throughout the experiment.

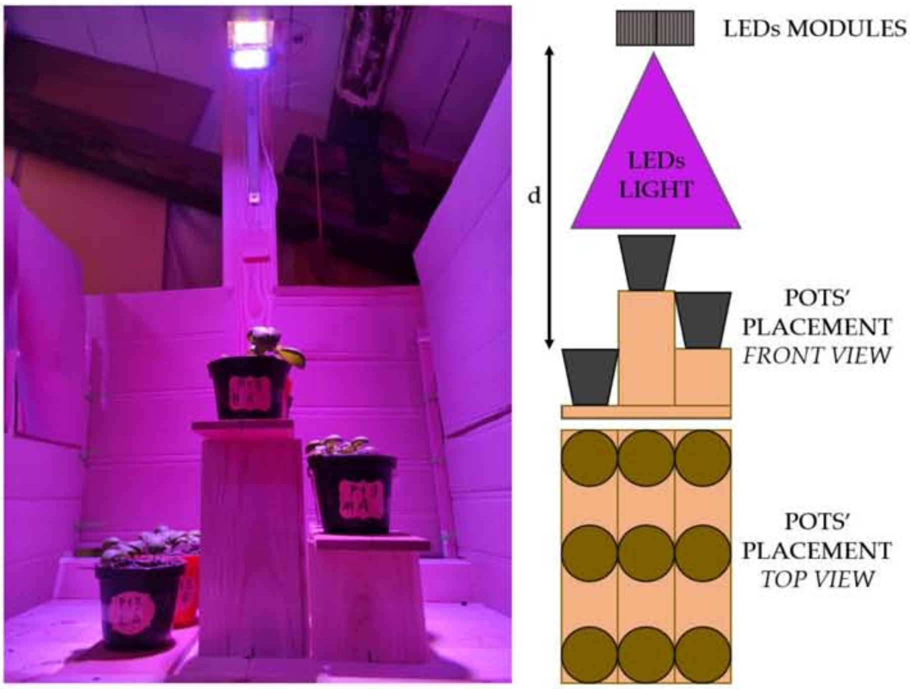


Figure 2. Experimental setup.

Accordingly, with the DoE, six different light combinations were needed. Each combination was obtained matching two LEDs modules among the ones reported in Table 3. The considered light combinations are summarized in Table 7, where the total calculated PPF and the composition in percentage for each type of LED are reported.

Table 7. Summary of LEDs composition for each considered light combination and corresponding Photosynthetic Photon Flux composition.

Light Combination's Code [1]	# of HR LEDs	# of DB LEDs	# of WW LEDs	PPF TOTAL [2] [μmol/s]	%PPF HR	%PPF DB	%PPF White
LR12	14	4	6	46.08	61.19	19.64	19.18
LR13	11	7	6	46.83	47.31	33.82	18.87
LR14	8	10	6	47.57	33.87	47.56	18.58
LR22	18	6	-	49.82	72.76	27.74	0
LR33	12	12	-	51.31	47.1	52.9	0
LR44	6	18	-	52.8	22.88	77.12	0

[1] Notation: LRxy means that the light combination has been obtained combining one module of type x with a module of type y (e.g., LR14 is the light combination obtained combining a LED module of type 1 with a LED module of type 4). [2] Obtained by the PPF's sum of the corresponding single PPFs shown in Table 3.

The same measurements conducted for each single module were repeated for each light combination by exploiting the same AS7341 low-cost spectral sensor at the three distances, *d*, considered in the experiments (i.e., 60, 70 and 80 cm respectively). The obtained results are summarized in Figure 3. In agreement with the theory, for a given light combination and a given distance *d*, the resulting light spectrum is, in first approximation, the sum of the spectrums of the two single LEDs modules used for the light combination. The reasons of the slight differences between theoretical and experimental results are twofold. The first one concerns a minimum misalignment between light source and sensor due to the manual positioning of the sensor itself conducted during the measurements. The second one, is due to a combination of two different aspects. From one side, each LEDs module has a beam angle of about 30 degrees. This means that the divergent angle associated with the modules, would cause the decrease in light intensity, which appears to be higher for samples placed at 70 and 80 cm than for those placed at 60 cm. On the other side, the sensor used has a front adapter with a diffuser that is hold in the right position by a mechanical fixture that could partially shadow the active area of the sensor in case of misalignment.

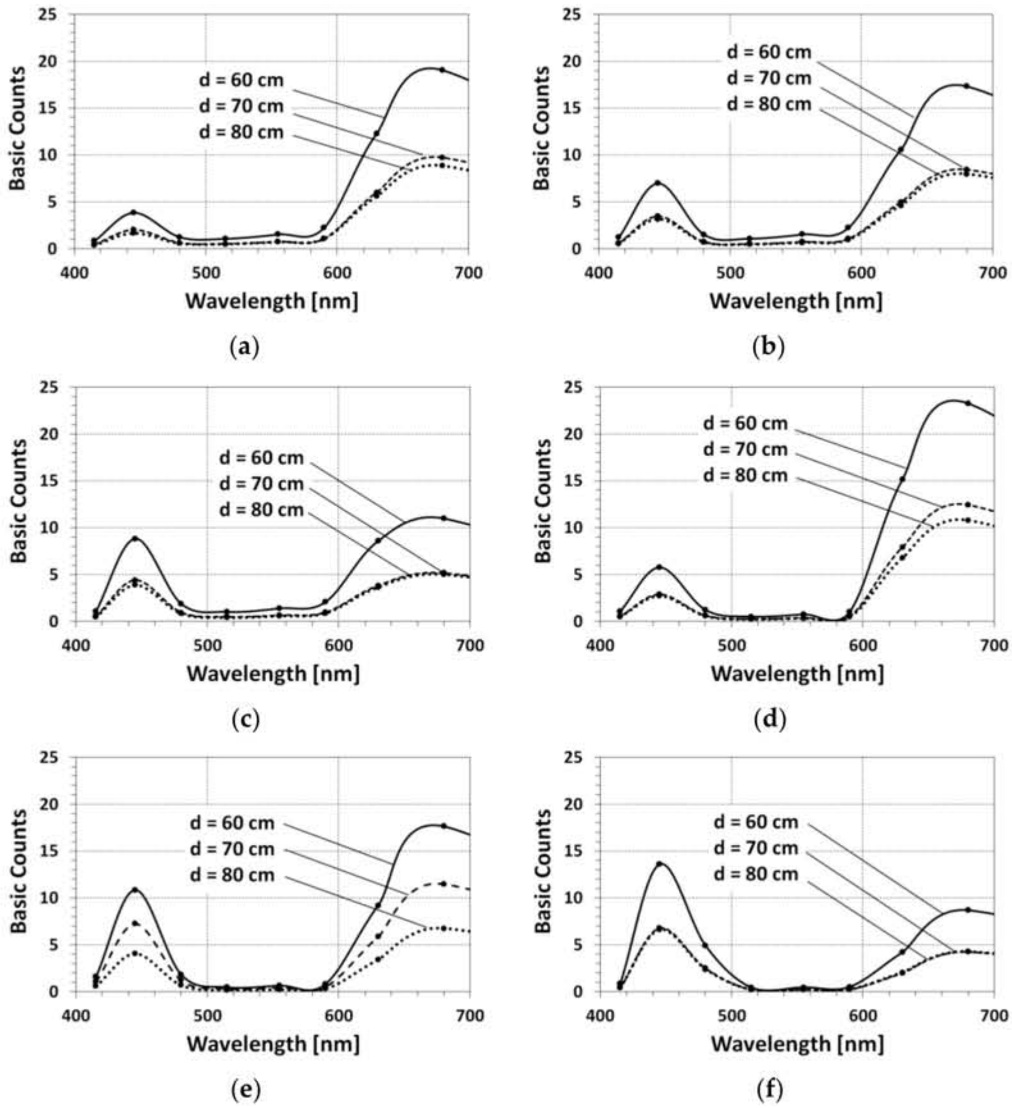


Figure 3. Light spectra of the 6 light combinations considered in the DoE, at the 3 different LEDs to plants distances, *d*. (**a**) LR12, (**b**) LR13, (**c**) LR14, (**d**) LR22, (**e**) LR33, (**f**) LR44.

2.4. Characterizations

A total of 14 responses were investigated to draw reliable considerations about germination and growth of basil. Germination potential of each combination was considered by measuring the Number of Plants and the Days needed for Germination. For the growth phase, at the conclusion of each trial, the following properties were measured for each plant: Wet mass, Dry mass, Height and Number of leaves. For each leaf, wet mass and area were measured. By adding up all the masses and areas of the same plant, the Total leaves mass and Total leaves area were calculated for each plant. Leaf wet mass and Average leaf area were calculated as well, (5), (6) formulae:

$$\text{Leaves wet mass} = \text{Total leaves mass}/\text{Number of leaves} \quad (5)$$

$$\text{Average leaf area} = \text{Total leaves area}/\text{Number of leaves} \quad (6)$$

Considering stem properties, average length, diameter, wet mass and dry mass were calculated for each plant. Finally, also the mass of water employed for irrigation during all the experiment was measured by measuring the weight of water employed for each test in all the period of trial. For masses, a laboratory balance (G&G GmbH, model PLC200B-C) with sensitivity ±0.001 g was employed and for heights a digital caliper (Borletti CDJB15-20 series) with resolution 0.01 mm, accuracy ±0.02 mm was used. For dry mass measurement, plants and leaves were dried at 80 °C for 24 h. Leaf area measurement was performed by scanning each leave at 400 dpi on graph paper and measuring using ImageJ software (version 1.52, NIH, Bethesda, ML, USA). Prior to scanning, leaves were cut at certain points to extend their full area on the paper and to better assess their area. Following these measurements, the LAI (Leaf Area Index), given by the ratio of Average leaf area to the area of the pot in which the plants had grown, and the SLA (Specific Leaf Area) index, given by the ratio of Average leaf area to Average leaf dry mass, were also calculated [4,16].

3. Results and Discussion

3.1. Preliminary Consideration

From a general and qualitative analysis of the results, germination occurred for almost all the basil seeds and any morphological or developmental abnormalities were observed, thereafter, all the tested light conditions resulted suitable for basil growth, even if with different performances. Figure 4 shows a visual comparison of the plants obtained at the final harvest of six different samples (Table 6), from which qualitative observation can be drawn and discussed. For each sample, all three repetitions were shown to stress the overall reliable repeatability of each experiment, considering that biological systems were investigated. Sample 6, 12 and 19 (Figure 4a–c) have in common the same LEDs combination (LR33) but are different from each other for the light-plant distance that is equal to 80, 70 and 60 respectively. In strong similarity, sample 2, 8 and 15 (Figure 4d–f) are representative of the same light combination (LR14) but with different light intensity, thereafter, moving from greater to smaller LEDs-plants distance. For both the sets, thereafter, independently of the LED combination, an increasing in plants height, number of leaf and leaf area can be observed by reducing placing the plants nearer to the artificial lights. In Figure 4c,f plants' height is clearly over the reference of 12 cm (also considering the pot), whereas in Figure 4b,e plants' height is approximately equal to 12 cm and in Figure 4a,d the overall plants' height is below the value of reference. Nevertheless, regarding the evaluation of the different LEDs combination from a qualitative point of view, is not possible to clearly detect any difference, even if the set of samples employing LR14 combination (Figure 4d–f) seems to better enhance plant growth, with respect to the other set of LEDs. Only observing sample 15 (Figure 4f) it is possible to suppose that this light condition (LR14 at a distance of 60 cm) seems to be the best to promote the basil height and leaf area, among those considered in Figure 4 but further confirmation must be drawn from the statistical analysis. Considering that other variables must be evaluated, such as more different light combination and more growth performance parameters, a statistical approach must be further considered to draw statistically reliable conclusion and to identify mathematical correlations.

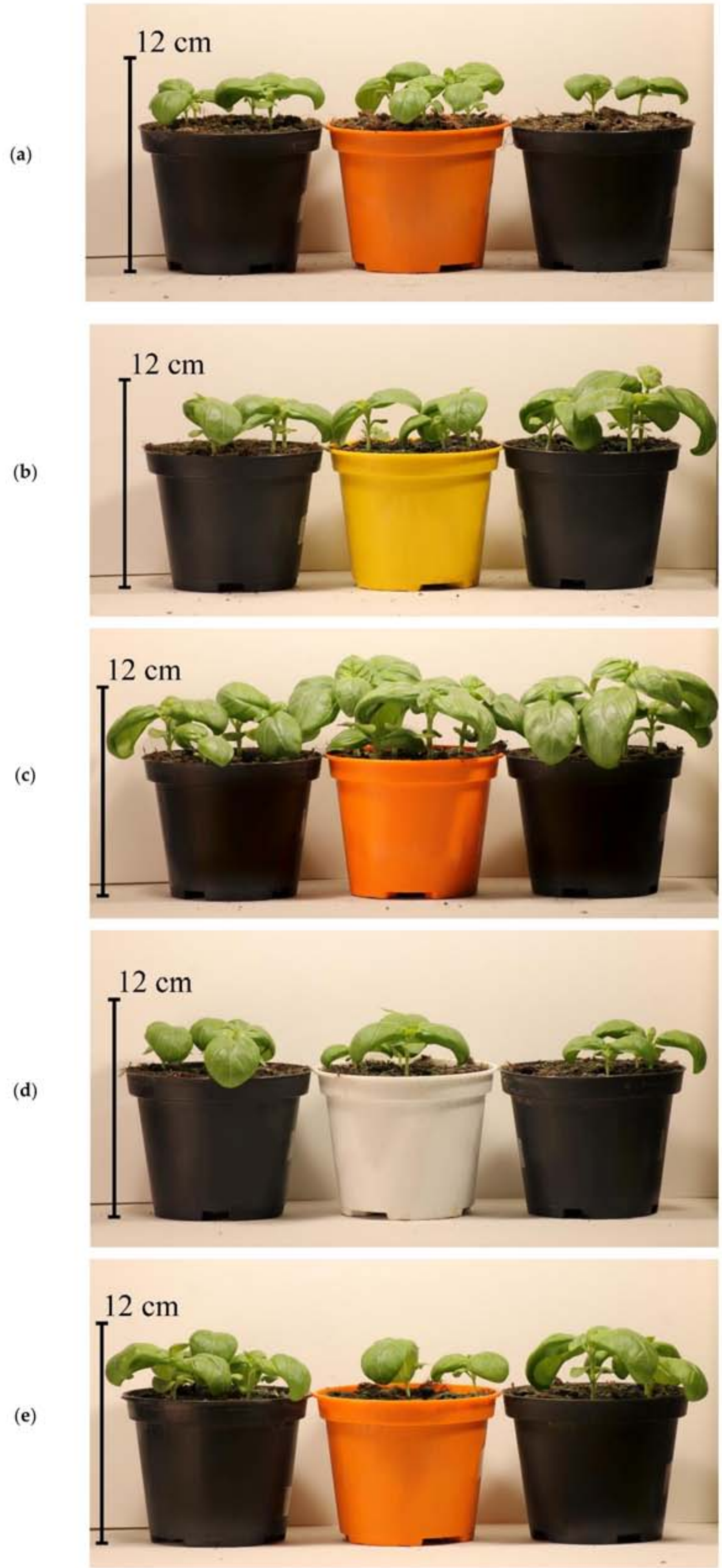


Figure 4. *Cont*.

(f)

Figure 4. Basil plants at the end of the growth test: (**a**) Sample 6, (**b**) Sample 12, (**c**) Sample 19, (**d**) Sample 2, (**e**) Sample 8, and (**f**) Sample 15. All the sample numbers are referred to in Table 6.

In addition, a control group of plant grown without any type of light has been tested, obtaining a not significant number of plants to derive any specific comparison among data. For the control group, only the plants' height was detectable and was measured equal to 2.03 ± 0.62 mm. This result suggests that LEDs light combinations investigated in this study play a valuable role as a different source of enlightenment from daylight to improve the basil growth performance.

3.2. Anova Analysis

A total of 14 responses were evaluated with statistical methods, and the values considered for each run were the average values of the measurements among three repetitions of the same run (Table 8). As previously stated, with the aim to evaluate only artificial lights effects, all the other parameters were kept as constant, thereafter, temperature and humidity were controlled during all the experiments to avoid environmental conditioning. Temperature and humidity were kept almost constant near 19 °C and 60% respectively, with restrained variation around 5% due to the employment of an indoor environment not perfectly conditioned.

The normal distribution of the residuals, as well as their homogeneity, was analyzed (data not reported) for each response, confirming that each mathematical model derived can be used to explore the region of interest. ANOVA results are presented in Table 9 and Figures 5–10. Models correlating the different lights conditions (in single or interaction) to growth and germination performance are significant as confirmed by the p value < 0.05 for all the responses, indicating that there are any factors that are significant and unknown for the data variation. Moreover, the curvature is not significant, suggesting that the central points can be treated as additional data in the regression model, augmenting the design plan. R^2 and Pred-R^2 (Table 9) confirms the overall good fit of the data, with only two responses with unacceptable quality, *Number of plants* and *Days for germination* having $R^2 < 0.45$. These two responses are mainly related with the germination phase that according to literature is the more critical to model [28]. However, a particular good fitting of the model is shown by *LAI*, *Dry mass*, *Stem dry mass* and *Water* responses with values of R^2 equal or above 0.90. The great majority of the resulting models allowed to describe the relationships among light conditions and the measured response. Considering the models' equations (Table 9) not only the *Distance* plays a valuable role to define the growth performance, as already assessed by the preliminary observation, but also the presence of the *White* LED and the proportion among hyper red and deep blue must be considered for most of the response. Thereafter, all the independent variables investigated in this study are relevant to define the growth performance of basil. This result is a new finding with respect to a previous research in which LED lights effect on basil germination and growth were investigated in combination to NPK fertilizer obtained in a circular economy perspective [28].

Table 8. Results.

Sample	High [mm]	Wet Mass [g]	Dry Mass [g]	Number of Leaves	Stem Length [mm]	Stem Wet Mass [g]	Stem Dry Mass [g]	Leaves Wet Mass [g]	Stem Diameter [mm]	Number of Plants [mm]	LAI [%]	SLA [g/cm^2]	Water [g]	Days of Germination
1	64.705	2.0107	0.1977	4	51.577	0.387	0.029	1.61	2.397	4	2.745	335.714	500	14
2	36.555	0.9905	0.0725	3	27.52	0.203	0.014	0.77	1.745	4	1.496	510.03	460	9
3	47.912	1.4652	0.1272	4	35.467	0.2462	0.0165	1.2	2.215	4	2.131	382.482	440	9
4	34.163	0.8396	0.0696	3.33	25.54	0.171	0.013	0.66	1.803	3	1.081	508.694	420	8
5	30.227	0.4682	0.0378	2	21.737	0.111	0.009	0.34	1.372	4	0.825	585.481	400	15
6	35.732	0.8258	0.0665	3	25.297	0.168	0.012	0.72	1.747	4	1.357	497.334	420	8
7	58.768	1.6572	0.1426	4	44.514	0.305	0.018	1.38	2.109	5	3.081	427.778	480	10
8	70.392	2.5177	0.2727	5	59.105	0.478	0.061	2.01	2.405	4	3.388	323.199	520	9
9	24.45	0.4826	0.0383	2	16.913	0.11	0.009	0.36	1.536	3	0.587	527.777	410	11
10	33.03	0.9076	0.0733	3.33	23.03	0.16	0.009	0.74	1.753	3	1.092	451.222	460	11
11	34.76	0.8867	0.0745	2.25	21.1	0.154	0.009	0.73	1.945	4	1.54	470.328	440	12
12	25.557	0.5888	0.0495	2	17.947	0.133	0.01	0.45	1.6	4	0.932	466.9	420	9
13	47.84	1.9922	0.1854	4	32.912	0.295	0.026	1.69	2.564	5	3.18	356.697	480	12
14	38.24	1.1395	0.0817	3	24.73	0.214	0.014	0.91	1.982	4	1.725	500.773	420	17
15	70.368	1.6722	0.1712	4.4	54.75	0.376	0.032	1.28	2.148	5	2.967	400.627	480	10
16	29.103	0.5283	0.0406	2	18.373	0.121	0.012	0.4	1.673	3	0.67	616.623	400	13
17	35.016	0.8626	0.0673	3.33	22.833	0.154	0.011	0.69	1.883	3	1.069	508.54	400	7
18	40.2	1.2056	0.1049	2.8	28.422	0.221	0.0154	0.97	2.056	5	2.387	427.278	440	11
19	56.042	2.2845	0.2596	4	46.81	0.436	0.0516	1.83	2.522	5	3.678	328.818	480	9
20	51.716	1.7271	0.1696	4	36.866	0.27	0.019	1.44	2.35	3	1.85	321.679	440	8

Table 9. ANOVA results.

Response	R^2	Pred-R^2	Equation White = YES	Equation White = NO
Number of plants	0.44	0.32	–	
Days for germination	0.12	0.07	–	
Height	0.76	0.70	=28.7796 + 15.2203 * Distance	
Wet mass	0.88	0.83	=0.6871 + 0.4952 * Distance	=0.4957 + 0.8752 * Distance
Dry mass	0.92	0.82	=−0.0024 − 0.0093 * Distance + 0.003 * HR:DB + 0.0320 * Distance^2	=−0.0169 + 0.0346 * Distance + 0.0035 * HR:DB + 0.0320 * Distance^2
LAI	0.93	0.91	=0.9884 + 0.2941 * Distance + 0.3199 * Distance^2	=0.8569 + 0.6771 * Distance + 0.3199 * Distance^2
SLA	0.81	0.67	=690.7412 − 82.6704 * Distance − 7.9299 * HR:DB + 0.0863 * HR:DB^2	=653.9108 − 82.6704 * Distance − 7.9299 * HR:DB + 0.0863 * HR:DB^2
Number of leaves	0.77	0.63	=3.3393 + 0.6230 * Distance − 0.0133 * HR:DB	=2.7819 + 1.1666 * Distance − 0.0135 * HR:DB
Leaves wet mass	0.89	0.84	=0.5612 + 0.3854 * Distance	=0.3784 + 0.7295 * Distance
Response	**R^2**	**Pred-R^2**	**Equation White = YES**	**Equation White = NO**
Stem length	0.86	0.76	=20.8343 + 8.1046 * Distance + 0.0086 * HR:DB − 0.1563 * Distance * HR:DB 6.6094 * Distance^2	
Stem wet mass	0.87	0.81	=0.1941 + 0.0280 * Distance − 0.0011 * HR:DB + 0.0473 * Distance^2	
Stem dry mass	0.94	0.88	=−4.4016 − 0.0272 * Distance − 0.0006 * HR:DB − 0.0058 * Distance * HR:DB + 0.3453 * Distance^2	=−4.5812 + 0.3701 * Distance − 0.0006 * HR:DB − 0.0058 * Distance * HR:DB + 0.3453 * Distance^2
Stem diameter	0.83	0.76	=1.7462 + 0.2290 * Distance	=1.5396 + 0.4969 * Distance
Water	0.90	0.83	=404.5918 + 39.6258 * Distance + 0.0367 * HR:DB	=436.0408 + 39.6258 * Distance − 0.5333 * HR:DB

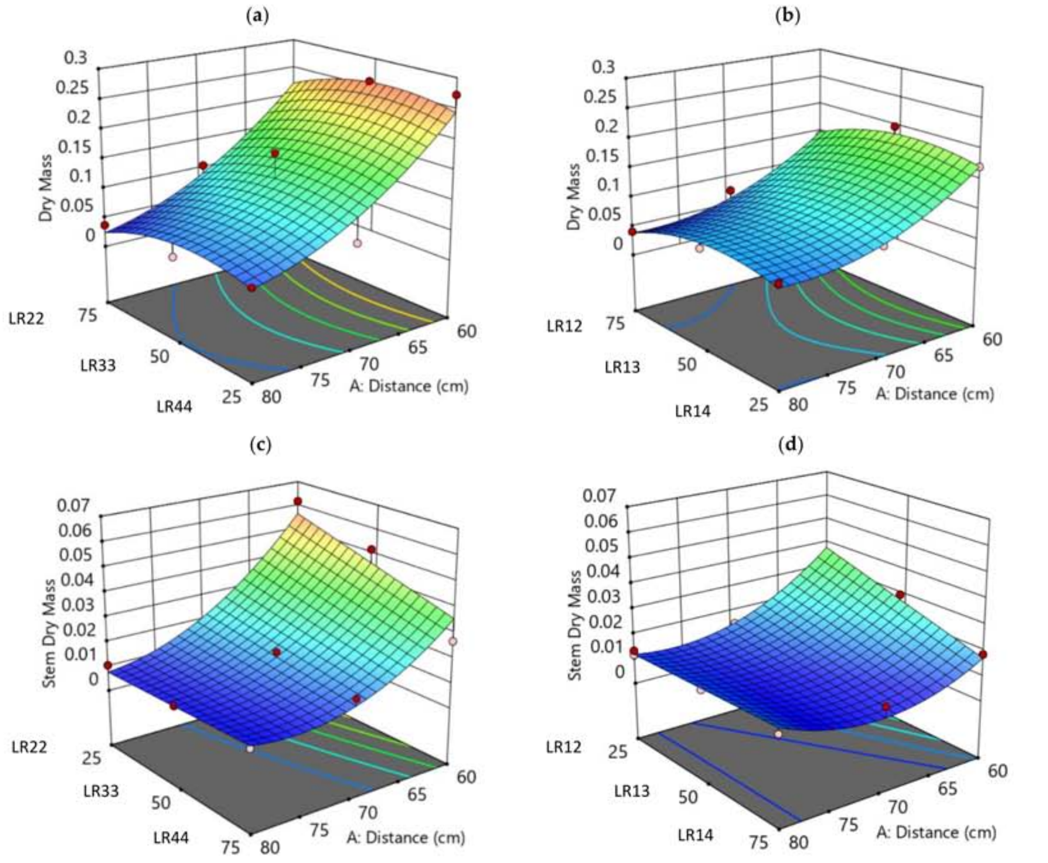


Figure 5. 3D surface contour plots of two different responses: dry mass, without (**a**) and with (**b**) white LEDs; stem dry mass, without (**c**) and with (**d**) White LEDs. Reference to Table 7 for light combination code.

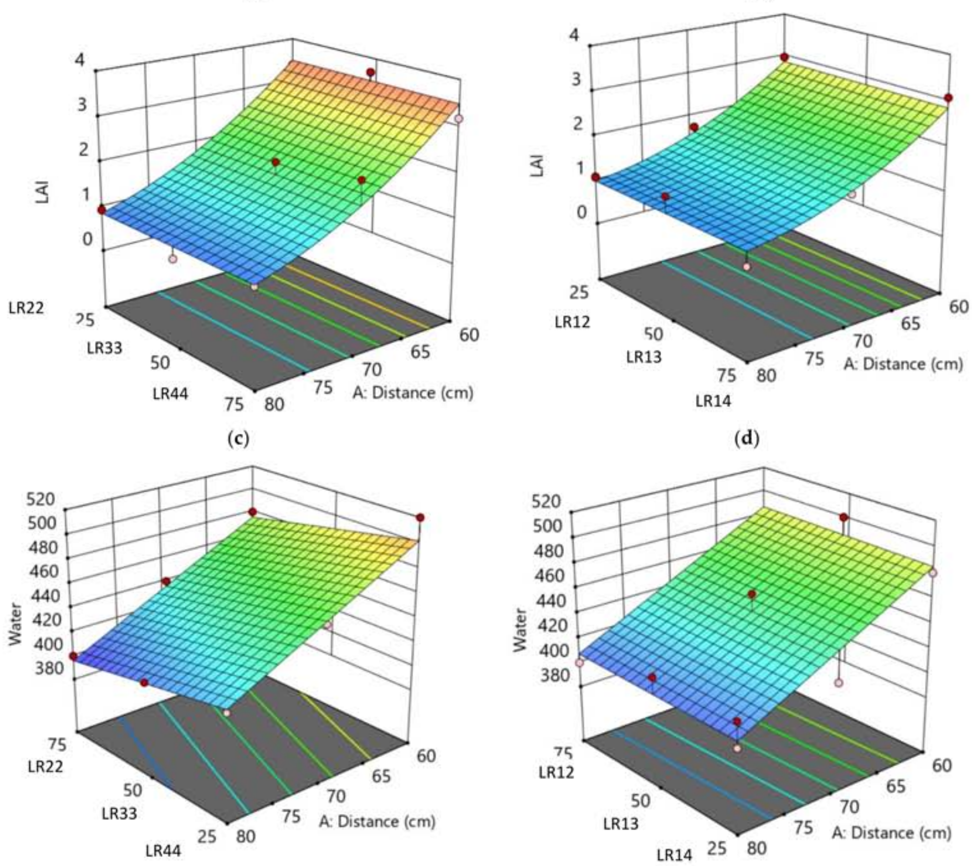
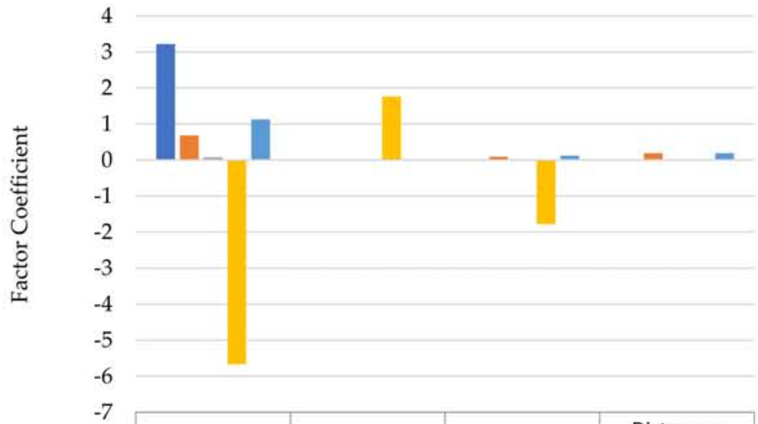


Figure 6. 3D surface contour plots of different responses: LAI, without (**a**) and with (**b**) white LEDs; water, without (**c**) and with (**d**) white LEDs. Reference to Table 7 for light combination code.

(a)

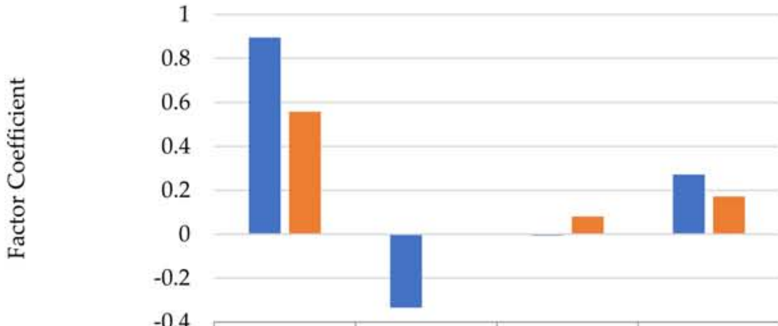


(b)

	Distance	HR:DB	White	Distance - White
Heigh	3.22	0	0	0
Wet mass	0.6853	0	0.0943	0.19
Dry mass	0.0768	-0.011	0.0147	0.022
SLA	-5.67	1.76	-1.78	0
LAI	1.13	0	0.1257	0.1915

Figure 7. Effects sizes of the most relevant independent variable on several significant responses related to the overall plant. (**a**) Graphical trend and (**b**) numerical coefficients (error bar = 0.05%, too small to be visible on the graph).

(**a**)

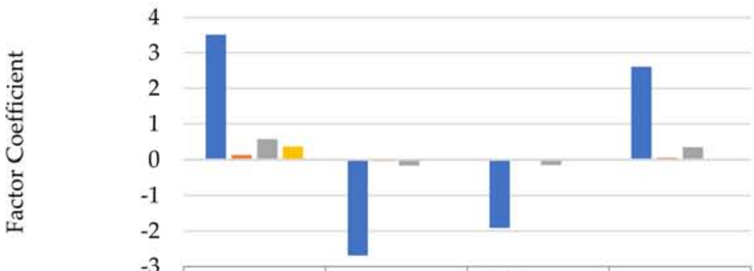


(**b**)

	Distance	HR:DB	White	Distance - White
Number of leaves	0.8949	-0.3343	-0.0069	0.2718
Leaves wet mass	0.5575	0	0.0806	0.172

Figure 8. Effects sizes of the most relevant independent variable on several significant responses related to leaf. (**a**) Graphical trend and (**b**) numerical coefficients (error bar = 0.05%, too small to be visible on the graph).

(**a**)

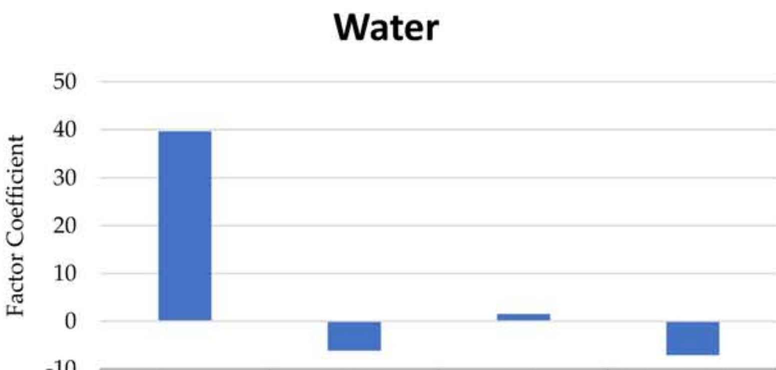


(**b**)

	Distance	HR:DB	Distance - HR:DB	Distance^2
Stem lenght	3.51	-2.69	-1.91	2.61
Stem wet mass	0.1227	-0.0299	0	0.0473
Stem dry mass	0.5695	-0.1617	-0.1463	0.3453
Stem diameter	0.363	0	0	0

Figure 9. Effects sizes of the most relevant independent variable on several significant responses related to stem. (**a**) Graphical trend and (**b**) numerical coefficients (error bar = 0.05%, too small to be visible on the graph).

(**a**)



(**b**)

	Distance	HR:DB	White	HR:DB - White
Water	39.63	-6.21	1.47	-7.13

Figure 10. Effects sizes of the most relevant independent variable on the response water. (**a**) Graphical trend and (**b**) numerical coefficients (error bar = 0.05%, too small to be visible on the graph).

In Figures 5 and 6, 3D surface graphs representing graphically the calculated models with $R^2 > 0.9$ are shown. In Figure 5, the two responses related with dry mass (*Dry mass* and *Stem dry mass*) were reported demonstrating for first how much the mathematical model changes by employing (figures on the right) or not (figures on the left) *White* LEDs. Both the responses are favored by the absence of the *White* LED. In addition, it is possible to see clearly that the *Distance* plays the most significant role for both the responses, *Dry mass* and *Stem dry mass*, even if for the first a not negligible effect is due to the proportion among hyper red and deep blue. To promote the dry mass, not only the lowest *Distance* must be selected but also a 1:1 proportion among hyper red and deep blue LEDs that correspond to light combinations LR33 and LR13.

Different behavior can be observed for the responses *LAI* and *Water* (Figure 6). *LAI* (Figure 6a,b) is mainly affected by *Distance* and only a slight effect is observed for the *White* LED. Conditions that optimize this parameter are lower *Distance* and the absence of *White* LED, independently of the ratio among hyper red and deep blue (Light combination LR22, LR33, LR44). Particularly interesting is the evaluation of the *Water* necessary to irrigation over the experimentation (Figure 6c,d) because this one is the only parameter that we desire to minimize to save an important natural resource such as water. As expected, the lower *Distance* increases the water consumption, as that more near the lights, the higher the need of water for plants due to the artificial light heating. The *White* LED alone is not particularly relevant for this response as can be seen by comparing Figures 6c and 6d, because almost the same range of mass of water is necessary; nevertheless, a different shape of the response surface is detectable as the interaction with the other parameter is reliable. Without white LEDs, the light combination LR22 must be preferred to reduce the amount of water to employ (Figure 6c), whereas no particular difference can be noticed among the LEDs combinations LR12, LR13, LR14 if the same distance is applied. This result suggests that the *White LED* addition must be well considered to reduce the water consumption. At the same time, the lower *Distance* that seems to promote that certain growth parameters (Dry mass, LAI and Stem dry mass) should be increased with the aim to minimize the water consumption.

Thereafter, it is interesting to observe quantitatively how much each independent variable, in single or interaction, is capable to affect a specific property, considering each elaborated model. This can be observed from Figures 7–10, where size effects were reported for each significant response, by grouping them for specific basil parts.

Regarding responses related to the overall plant (Figure 7), *Distance* plays absolutely the main role for all the responses; nevertheless, a negative effect is reported for *SLA*. This means that the same *Distance* that is capable to promote some properties (e.g., *Heigh* or *LAI*) is also responsible for a reduction of the *SLA* parameter. As shown from Figure 7, for *SLA* parameter the reduction due to an increasing distance can be recovered by increasing the *HR:DB* ratio, thereafter introducing more hyper-red LEDs with respect to deep blue.

Considering the two responses related to leaf growth (Figure 8) a reliable and positive effect is associated to *Distance* and *White* LEDs employment in single and in interaction. However, the effect of the ratio *HR:DB* is negligible for *Leaves wet mass* and strongly negative for *Number of leaves*, suggesting the need to employ the lights combination LR44 to promote the leaf number, as confirmed by a previous study [28].

Evaluating the responses related to stem properties (Figure 9), again it is possible to detect a strong influence of the *Distance,* as it has a strong effect as single factor and in interaction with *HR:DB*. Even if in this case, a competitive effect emerged related to the response *Stem length*, that is affected in a positive way from *Distance* in single factor but a negative way when its interaction with *HR:DB* is considered, leading to a restrained range of light combination–distance conditions capable to promote this property. A similar situation describes *Stem dry mass* and *Stem wet mass* but with restrained size effect with respect to *Stem length*. Thereafter, conditions that will promote stem growth should be particularly well-tailored to match all these competitive effects.

Finally, the estimated effect size of *Water* (Figure 10) indicates that the variables that should be emphasized to reduce its consumption are *HR:DB*, as it affects Water negatively, and its interaction with *White* LED, for the same reason. However, *Distance* must be severely reduced to decrease the Water consumption as well. This means that the employment of the light combination LR22 or LR12 at a distance equal to 60 cm should be preferable, as argued considering Figure 6c,d.

As shown from these results, it is hard to establish a light condition that can be favorable for all the responses; in particular, is not clear if the overall influence of the white LED is beneficial or detrimental for the basil growth. As suggested in the literature, possible overlapping effect of the white LED with the others LED light that are more absorbable by the plants can have a negative effect on the plants' growth, but this effect depends on the parameters that are investigated [24]. Thereafter, it is necessary to collect all the derived models in one, called desirability function, by balancing them, considering each goal and importance with respect to the overall aim of the research.

The desirability function conditions employed in the present work were shown in Table 10. Number of plants and days for germination responses were discarded from this part of the analysis due to theirs poor R^2 and Pred-R^2, indicating a low fitting and predictive power. The other responses were evaluated through the objectives in Table 10 with the aim to promote the overall plants' best growth with the most possible restrained amount of water.

Table 10. Desirability function conditions.

Responses	Goal	Importance
Height	maximize	4
Wet mass	maximize	5
Dry mass	maximize	4
LAI	maximize	4
SLA	maximize	5
Number of leaves	maximize	4
Leaves wet mass	maximize	3
Stem lenght	maximize	3
Stem wet mass	In range	3
Stem dry mass	In range	3
Stem diameter	maximize	2
Water	minimize	5

As shown in Figure 11, results of the desirability function calculation indicate that the light conditions that better fit the overall objectives indicated in Table 10 are a distance equal to 60 cm and the employment of the LR22 light combination (green area of the graphs). This condition has a desirability value equal to 0.618956 as shown in Figure 11a, and it is the highest one of the overall light conditions investigated in this study. This result agrees with the fact that most of the responses are strongly promoted by a distance equal to 60 cm and the fact that water consumption is reduced avoiding white LEDs light. In addition, this result agrees with previous literature on hyper red effect on basil growth [40,41] However, the blue area of the graphs indicated all the lights combinations that should be avoided, to promote the basil growth. By employing or not white LED, this area changes; without white LED (Figure 11a), the blue area of the graph in mainly due to distance > 75 cm, and by employing white LED (Figure 11b) this area results wider, as the interaction of distances between 65–78 cm and hyper red prevalence in proportion to deep blue (LR 12).

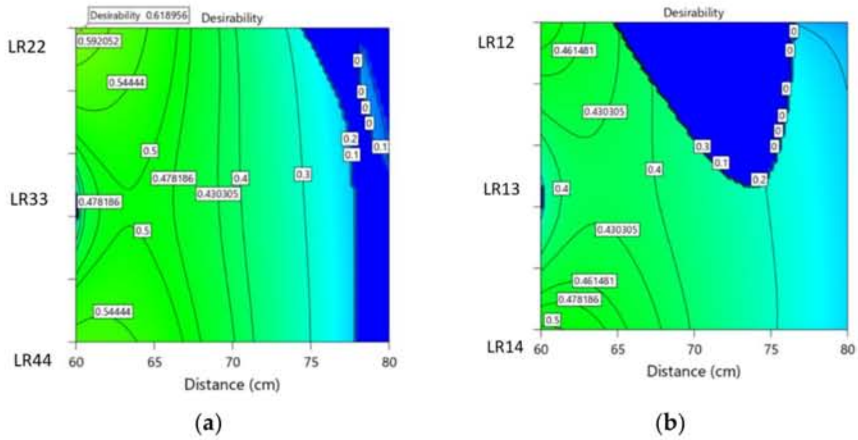


Figure 11. Desirability 2D contour plot without (**a**) and with (**b**) White LED light.

4. Conclusions

In this study, it has been demonstrated that the implementation of various combination of LED lights is generally favorable to basil (*Ocimum Basilicum)* germination and growth. Applying a design of experiments approach, mathematically reliable information has been derived concerning a synergic effect among LED light type and light–plant distance. It has been calculated that by avoiding white LED light, better performance in terms of crop yield enhancement can be reached. Furthermore, LED light combinations, involving hyper red and deep blue light in 3:1 proportion, results the best by considering the desirability function, which includes all the requirements to be satisfied for the overall basil growth. In addition, the distance among plants and light plays a key role; the shorter distance investigated (60 cm) is advisable for the basil growth, even if attention must be paid to water consumption, thereafter a plants-light distance among 65 cm is suggested. The present study has been focused on the influence of different factors on basil growth considering only the final harvest of the plant; but in a future, perspective data at different time of the basil growth can be collected. In this context, the generated models in different times of the plants' growth can be employed in artificial intelligence-driven systems to apply automatically specific light combination or distance in a particular moment of the plants' growth, to further promote the growth and the health of the plants.

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