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Quantitative Calculation of the Most Efficient LED Light Combinations at Specific Growth Stages for Basil Indoor Horticulture: Modeling through Design of Experiments

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Featured Application: The specific application of this work is related to basil cultivation in indoor horticulture, and is devoted to promote basil growth by employing optimized LED light recipes for each specific growth stage.

Abstract: Indoor farms are a promising way to obtain vegetables in standard quantity and quality. As opposed to previous studies, this study attempts to calculate optimized LED light conditions for different growth stages (five-days time step) of basil (*Ocimum basilicum*) to enhance its indoor growth through a statistical approach. Design of Experiments (DoE) was used to plan a limited number of experiments (20) and to calculate quantitatively the effect of different light recipes on four responses: the number of plants, their height, the Leaf Area Index, and the amount of water used. Different proportions (from 25% to 77%) of Hyper Red (660 nm) and Deep Blue (451 nm), intensities in terms of LEDs–plant distance (60, 70 and 80 cm), and the addition of Warm White (3000 K) LEDs were considered as independent variables. The obtained models suggest that a light recipe tailored for every growth step in the plant's life is beneficial. Appropriate LEDs must be carefully chosen at the beginning of growth, whereas distance becomes relevant at the end. This is confirmed by the results analysis carried out at the end of an additional growth test where the optimal light recipe extracted from the DoE's results were used.

Keywords: mathematical modeling; artificial light; vertical farms

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1. Introduction

Globally, strong efforts to develop new and more efficient agricultural solutions, with respect to conventional farming, must be made for several reasons. Firstly, the production of primary crops, that has had an increase equal to 53% between 2000 and 2019, will further increase [1]. Secondly, nowadays, agriculture is already facing pressure from climate change as current and conventional systems contribute actively to the release of pollutants into the atmosphere, such as GHG emissions and 10.7 billion tons of carbon dioxide equivalent in 2019 [2,3]. In addition, the progressive reduction of arable lands questions the conventional field model since phenomena, such as urbanization and desertification, are expected to comprise between 1.8% and 4.6% of global lands by 2100 [4].

Finally, as the loss in crop yield is expected to range from 10% to 50% by 2030, it is pivotal to search for and optimize new agricultural models [5].

Farming models that differ from field farms in their location, such as urban areas, integrated into buildings, and in conditioned or unconditioned closed environments, have been discussed in the literature [6,7]. Among them, indoor farms have many advantages, as their efficiency does not depend on seasonality and their usage does not exploit land area. However, a huge amount of energy is required due to high operating costs mainly related to lighting, but also to temperature and humidity control, and highly specialized labor [7–9]. In this context, huge effort must be devoted to developing automated systems with low energy consumption and possibly integrated with renewable resources such as solar or wind energy, according to the present European policy regarding energy consumption savings [10]. Among these, vertical farms have the most high-tech architecture, as they are fully indoor and often based on hydro or aeroponics systems [7,9]. In vertical farms, light is provided only by artificial systems, mainly constituted by LED (Light-Emitting Diode) modules, tunable, and with a relative low energy demand under controlled conditions [11]. In fact, LEDs have become the main source of artificial light in indoor farms thanks to higher performance with respect to other artificial lights, e.g., HPS (High-Pressure Sodium) lamps [12]. LEDs have also a narrow wavelength band, useful for tunable light recipes that can be variated according to plants' needs [11].

Nevertheless, the optimization of artificial lighting in vertical farms is essential for their energetic and economic viability, as lighting costs could reach 80% of the electricity demand of a vertical farm [13]. In fact, it has been demonstrated that a clever employment of light, e.g., use of an intermittent light system that is implemented considering variation in electricity prices, could allow a cost reduction of nearly 22% [8]. However, this saving must be obtained without losing the quality or quantity of the final yield. In this sense, the implementation and maintenance of good sensors used for indoor agriculture are also pivotal to enhance energy use and economic return for growers [14].

Furthermore, current knowledge of plant growth is based mainly on natural sunlight [10,15]. Therefore, there is not enough knowledge on the interaction between plants and artificial light, which would be essential to optimize both the quantity and quality of crop yield in indoor and vertical farms. Current studies separately consider different aspects of indoor conditioned farms, in order to improve the system's efficiency: the modeling of plants' evapotranspiration [16], the optimization of the environmental temperature regulation and measurement [17], and the effect of light intensity and wavelength [18,19]. In addition, it must be noted that every kind of crop follows its own growth process, so every type of plant requires specific studies which define tailored growth conditions, also considering the different growth stages.

Among the different plants, basil (*Ocimum basilicum*) is an aromatic herb valuable in the food and cosmetic industries, while its extracts are useful in medicine [20]. Basil is suitable for indoor farming for many reasons: it grows vertically but with a limited extension, has a richer flavor when grown in indoor conditions in respect to open field conditions, and has a short life cycle that allows its harvest several times a year [21,22]. Several works have investigated the influence of indoor environmental variables on the growth of basil plants, but often LEDs are used in addition to natural light [20,22]. Other works considered the effect of only artificial light on basil growth parameters and highlighted the usefulness of intermittent lighting, which allows basil growth with optimized use of energy; however, a quantitative calculation of different wavelengths of light and new lightning durations remains an open question [8]. Pennisi et al. studied the optimal PPFD (photosynthetic Photon Flux Density) for the indoor cultivation of basil, ranging from 100 to 300 μmol m^{-2} s^{-1} with a constant photoperiod of 16h d^{-1}, using red and blue light in a fixed ratio (Red/Blue = 3), finding that the optimized radiation intensity was 250 μmol m^{-2} s^{-1} [23]. Regarding the specific light recipe, the great majority of works are devoted to monochromatic LED lights and only a few have studied interactions between different wavelengths, among them: Piovene et al. investigated the physiological and phytochemical variations of basil in response to different ratios of blue and red light, finding that a Red/Blue ratio equal to 0.7 guaranteed the best results [24]; Jensen et al. demonstrated that spectral manipulation of the grow light can produce relevant effects on post-cultivation performance of chilling sensitive plants, and a ratio between Red and Green LEDs equal to 80:20 was suggested [25].

Main Contributions of This Work

While most of the previous studies highlight the viability of the vertical farm model, only a few works try to find solutions to very important aspects in real world scenarios, such as optimization of resources (water, fertilizers, etc.…) and minimization of operational and energy costs. This can be achieved by introducing both customized light recipes and sensor systems to monitor the plants during the whole growth cycle.

In this sense, a work related to the present research is the one from Barbi et al. [26], in which the effect of different light recipes composed of uncommon LED lights (Hyper Red, Deep Blue and White) on the growth performance of basil was evaluated through statistical methods. In this related work, the better light recipe for basil growth was indicated as follows: ratio HR:DB = 3:1, distance equal to 65 cm and exclusion of White LEDs. For the present work the same experimental conditions were kept, such as type of soil or type of LEDs, but in contrast to this previous work, the basil growth was examined not only at the end of the growth time, but also at several intermediate times, in order to calculate optimized LED light recipes for each time period [26].

In fact, the main hypothesis of this study is that basil needs different LED conditions at different stages of its growth to promote its overall growth efficiency in vertical farms, since in others studies the fact emerges that the addition of White LEDs has a relevant influence on the fresh and dry weights of basil and other species such as broccoli, cabbage and potato [27–29]. In addition, as opposed to the great majority of the studies found in the literature, the Design of Experiments (DoE) techniques have been applied with two main purposes: (i) to rationally design and limit the number of experiments required in order to collect the data concerning basil growth, and (ii) to quantitatively calculate a tailored light recipe for every growth step in the plant's life (five-days each) through analysis of variance (ANOVA) and multivariate linear regression. In this sense, this work will merge experimental work for data collection with mathematical model calculation based on real data. Finally, the results of the present work, in contrast to the related work and previous literature, will generate specific mathematical models that can be employed to promote different canopy properties (alone or in combination with others) at different times of plant growth, thereafter, giving to the final user a great control in vertical farm cultivation. In fact, in this study, mutual interaction between variables, such as type of LED and intensities, is quantitatively estimated, in contrast to previous literature where variables were only considered separately.

2. Materials and Methods

2.1. Experimental Growth Test

Growth tests were performed in a controlled indoor environment using nine pots per test with a growing area of 50 cm^2 each. As growing substrate, Floradur B pot coarse universal potting soil was chosen, enabling the growth of basil without the need to add further nutrients (Producer: Floragard Vertriebs GmbH, Oldenburg, Germany). This substrate was already employed in a previous study, demonstrating that its main nutritional elements and physical properties are suitable for basil growth [30]. Five seeds of basil (*Ocimum basilicum*) of the "Genovese" variety (Producer: Magnani Sementi) were sowed in every pot (Figure 1a) and grown for 30 days at 19 °C and a relative humidity equal to 60% ± 5%. Water was added once every two days to fulfill the constant humidity, and was recorded as a result.

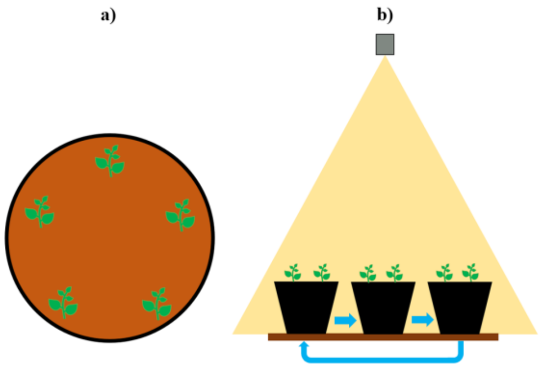


Figure 1. (**a**) Scheme of seedlings distribution in a single pot; (**b**) scheme of pots position under the same light conditions**.**

The used growth box was completely isolated from natural light. Artificial light was obtained using solely commercial LED modules specific for horticulture [31]. Each module comprised twelve OSRAM Oslon®SSL ThinGaN LEDs (UX:3). The modules had three different types of LEDs, i.e., Hyper Red (HR, wavelength = 660 nm, [32]), Deep Blue (DB, wavelength = 451 nm [33]), and Warm White (WW, color temperature = 3000 K, [34] arranged with different ratios of number of HR, DB and WW LEDs per module. This allowed to obtain a different light spectrum for each type of module. Table 1 summarizes the composition in terms of number of LEDs per type per module and corresponding Photosynthetic Photon Flux (PPF), obtained following the same procedure described in [26]. Accordingly, with the experimental plan explained in Section 2.2, all the light recipes tested were obtained using two modules each, i.e., 24 LEDs in total, in different combinations as summarized in Table 2, where the same notation used in Table 1 has been applied (e.g., 8HR:10DB:6WW means 8 Hyper Red LEDs, 10 Deep Blue LEDs and 6 Warm White LEDs). The LEDs were arranged in different combinations to obtain different HR:DB ratios, and the presence or absence of white light, as shown in Table 3 and as described in Section 2.2.

Table 1. Composition of the LED modules used in the experiments to create the light recipes considered in the experimental plan.

Module Code [1]	Total Number of LEDs	Total Number of HR LEDs	Total Number of DB LEDs	Total Number of WW LEDs	Total PPF [μmol/s]
5HR:1DB:6WW	12	5	1	6	21.17
9HR:3DB	12	9	3	-	24.91
6HR:6DB	12	6	6	-	25.66
3HR:9DB	12	3	6	-	26.40

[1] Module's coding: <# HR LEDs>HR: <# DB LEDs>DB: <# WW LEDs>WW.

Table 2. Calculation of the PPF for each considered module combination.

Combined Module's Code [1]	PPF HR [μmol/s]	PPF DB [μmol/s]	PPF WW [μmol/s]	Total PPF [μmol/s]	%PPF HR	%PPF DB	%PPF White
8HR 10DB 6WW	16.11	22.62	8.84	47.57	33.87	47.56	18.58
11HR 7DB 6WW	22.15	15.84	8.84	46.83	47.31	33.82	18.87
14HR 4DB 6WW	28.19	9.05	8.84	46.08	61.19	19.64	19.18
12HR 12DB	24.17	27.15	0.00	51.31	47.10	52.90	0.00
18HR 6DB	36.25	13.57	0.00	49.82	72.76	27.24	0.00
6HR 18DB	12.08	40.72	0.00	52.80	22.88	77.12	0.00

[1] Module's coding: <# HR LEDs>HR: <# DB LEDs>DB: <# WW LEDs>WW.

Table 3. Independent variables employed for the statistical analysis.

Factor	Type	Levels	Minimum	Central Point	Maximum
Distance	Numeric/Discrete	3	60 cm	70 cm	80 cm
HR:DB	Numeric/Discrete	3	25%	50%	75%
White	Categoric/Nominal	2	YES	-	NO

A photoperiod of 16 h/day was chosen. Pots were placed at three different distances from LEDs, namely 60, 70, and 80 cm between the light source and the top of pots, corresponding to three different light intensities. Distances were kept constant during growth, elevating LED modules. Pots at the same level were swapped every two days, to correct for possible differences in light intensity due to the different illumination angles (Figure 1b). In the experimental setup, the LED modules had an emission cone angle of about 30 degrees obtained by means of ad-hoc optical lens [35], and had a fixed power supply (i.e., a constant light intensity). Therefore, the only way to obtain different light intensities was to change the distance between plants and light source. From a practical point of view this meant that the larger the distance, the smaller the PPF emitted by the LED modules that reached the plants because the difference between the illuminated area and growth area was larger. In the proposed setup scenario, for each light recipe reported in Table 2, the average portion of PPF emitted by the LED modules that reached the plants could be approximated about 57%, 42% and 32% of the emitted one, for distances of 60 cm, 70 cm and 80 cm, respectively. These average values were obtained by using basic geometrical rules and also taking into account the LED module's lens efficiency (i.e., 88% for the used LED modules[35]).

2.2. Experimental Plan

In order to minimize the number of experimental tests needed to calculate the correlation between LED light recipes and basil growth, the Design Expert software (version: 13.0, developer: State Ease) was used to design the experimental data plan. Computer-aided experimental design is a strategy suitable for overcoming the strong limitations of the one-factor-at-time approach, with the aim to maximize the efficiency of the experimental observation when multiple variables are investigated. In fact, a limited and strictly necessary number of experiments can be planned to satisfy further statistical analysis and consequently the model calculation [36]. Three factors were considered as variables of the experimental observation: (i) distance, as the distance between plants and light source varied on three levels (60, 70 and 80 cm); (ii) HR:DB ratio, as the proportion between the number of Hyper Red LEDs and the number of Deep Blue LEDs varied on three levels (25%, 50%, 75%); and (iii) White, as the presence or absence of the white LEDs and a further addition of HR and DB in the 5:1 proportion, accordingly to Table 1. A two-level fractional factorial plan was considered with the inclusion of central points to minimize the number of tests and, at the same time, to enhance a lower average prediction variance. A total of 20 trials were planned, each consisting of three pots used as repetitions, to overcome possible limitations of the data reproducibility due to biological elements' observation. A summary of the data employed for the experimental plan calculation is shown in Table 3. The other variables occurring in the process and not specifically considered in this study, such as humidity and temperature, were kept constant during all tests, according to the procedure as explained in Section 2.1.

2.3. Characterizations

Measurements were taken every five days for all the shoots in every pot, namely 15, 20, 25, and 30 days after sowing. No measurements were taken before, as germinated plants were too small or too few for the single pot. Measured properties comprise the number of plants per pot (NoP), leaves area expressed as LAI index (LAI), average height calculated for every plant of each pot (Height), and amount of water given to every pot

(Water). Collected data were used as response variables in the DoE analysis; a total of 16 responses were analyzed, according to Table 4, as four properties were measured at four different basil growth steps.

Table 4. Responses analyzed through DoE.

Property	After 15 Days	After 20 Days	After 25 Days	After 30 Days
Height	Height_15D	Height_20D	Height_25D	Height_30D
Number of Plants	NoP_15D	NoP_20D	NoP_25D	NoP_30D
Leaf Area Index	LAI_15D	LAI_20D	LAI_25D	LAI_30D
Water	Water_15D	Water_20D	Water_25D	Water_30D

A digital caliper (Borletti CDJB15-20 series) was used to measure height, and the arithmetic average of all the plants of the same pot was taken as value reference for the experiment. Leaves area was measured performing image analysis on photos of pots, taken from a vertical point of view, using ImageJ software (Figure 2). The obtained leaves area was then used to calculate the Leaf Area Index (LAI) considering the pot growing area. Leaf Area Index is defined as the ratio between the area of leaves of plants and the area of soil under those plants [37]. Water was measured for every pot using a graduated cylinder.

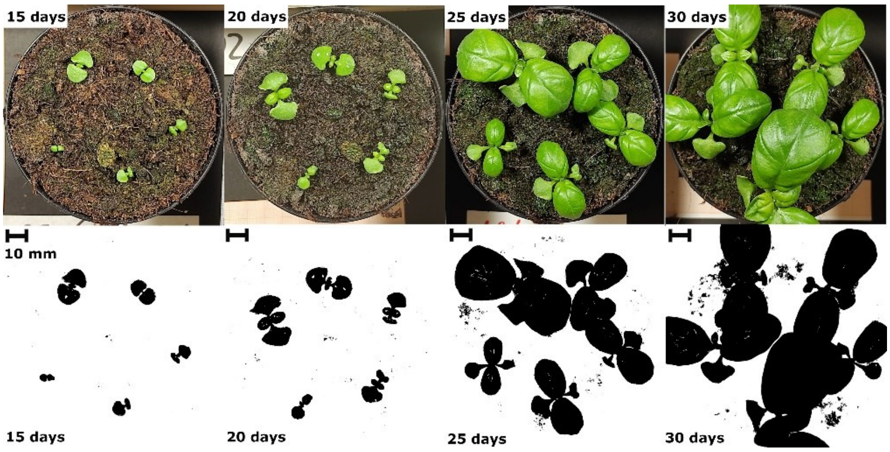


Figure 2. Example of the pictures taken for each pot in different phases of growth (top), and results of the elaboration performed to isolate and measure leaves area for the calculation of LAI (bottom).

2.4. Statistical Analysis

Analysis of Variance (ANOVA) was employed to perform the quantitative calculations of predictive models capable to define the basil growth in terms of single and synergic effects of the artificial light conditions. Preliminary conditions necessary to apply this approach were that the light conditions variable must be independent of each other and normally distributed in the chosen range [36]. In these conditions, by employing the F-test, it was possible to estimate if the variation among samples obtained in the same experimental condition was lower than the variation among all the samples [36]. A p-value lower than 0.05 was considered as threshold for factors and models significance, and for each significant factor the specific coefficient was measured, thereafter building up a mathematical model based on multiple linear regression, as shown in Formula (1):

$$Y = \beta\_0 + \beta\_1 X\_1 + \beta\_2 X\_2 + \ldots + \beta\_i X\_i \quad (1)$$

where Y is the response, $X(1 - i)$ are the independent factors and $\beta(1 - i)$ are the associated coefficients. The 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, as values nearer to 1 indicate a good quality of the fit. To better highlight the role of the main components on the final considered properties, response contour plots and mathematical equations were derived and discussed. In these graphs, areas characterized by hot colors, such as red or orange, represent areas of the plot where the response variable is at its higher values, and vice versa for cold colored areas. Finally, a global desirability function was calculated for each period of growth to provide the most desirable LED light combination, taking into account all the responses analyzed simultaneously. According to its definition, in the desirability function, each response is weighed according to its specific goal and importance (Table 5), evaluated depending on how and how much each response is relevant for the global purpose [36]. The desirability function range is from 0 to 1, where the lowest value (0) represents a completely undesirable combination of independent factors, and, conversely, the highest value (1) indicates a completely desirable or ideal combination of them.

Table 5. Desirability function parameters employed for each period of growth.

Response	Goal	Importance
Height	to maximize	4
Number of Plants	to maximize	4
Leaf Area Index	to maximize	3
Water	to minimize	1

3. Results and Discussion

In this section the obtained results are presented and discussed.

3.1. General Observation of the Experimental Tests

The results of all the measurements are reported in Table 6. From a rough evaluation of the collected data, it is possible to make some useful considerations for evaluating the benefit of the statistical analysis. First, it was noted that each response the data reported had a good variability; therefore, it is possible to suppose that the selected input factors may have some effect that can be calculated quantitatively on the responses. Nevertheless, it was not possible to identify a precise trend from a rough observation of the data collected for the same response. Comparing different responses, the data evolution of the same property, considering passing time, was generally consistent with expectations due to plant growth; for example, a general increase in the height and amount of water was observed by increasing the day of observation. Thereafter, in these conditions, a statistical analysis of the data could be performed, and was necessary for the quantitative calculation of the effects of the input factors on the selected responses.

Table 6. Results summary.

	Factors			Responses															
	1	2	3	Height (mm)				NoP				LAI				Water (g)			
Run	Distance (cm)	HR:DB	White	15D	20D	25D	30D	15D	20D	25D	30D	15D	20D	25D	30D	15D	20D	25D	30D
1	80	50	YES	8.009	10.450	14.996	24.517	3	3	3	3	0.033	0.072	0.139	0.347	200	270	320	350
2	70	25	NO	6.935	9.068	15.538	21.443	4	4	5	5	0.028	0.069	0.131	0.347	220	290	340	390
3	70	50	NO	6.611	10.660	15.467	23.953	3	3	3	3	0.022	0.053	0.119	0.346	180	270	320	370
4	60	25	YES	7.904	10.176	14.634	24.586	4	4	5	5	0.029	0.073	0.187	0.532	220	290	340	410
5	60	75	NO	6.361	10.782	15.253	21.420	4	4	4	4	0.022	0.058	0.131	0.428	220	290	340	410
6	60	50	YES	7.595	11.318	16.968	24.976	3	3	4	4	0.031	0.077	0.182	0.513	240	310	360	430
7	80	75	YES	5.792	8.221	12.284	20.442	2	2	2	2	0.009	0.023	0.060	0.122	180	250	300	330
8	80	25	NO	6.880	8.691	13.884	19.888	3	3	3	3	0.018	0.051	0.097	0.239	200	270	320	350
9	60	50	NO	6.974	11.133	17.990	26.348	4	4	4	4	0.038	0.095	0.231	0.638	200	290	340	410

10	70	50	YES	8.819	9.943	14.902	21.328	2	3	3	3	0.016	0.040	0.074	0.202	220	290	340	390
11	70	75	YES	6.740	9.383	12.352	19.144	4	4	4	4	0.018	0.046	0.096	0.258	200	270	320	370
12	60	25	NO	6.758	9.588	17.101	24.102	3	3	3	3	0.030	0.072	0.164	0.489	240	330	380	450
13	80	75	NO	6.402	9.921	13.610	21.017	4	4	4	4	0.021	0.052	0.098	0.245	180	250	300	330
14	70	25	YES	8.457	11.412	17.987	24.712	3	3	3	3	0.035	0.072	0.131	0.332	180	250	300	350
15	80	25	YES	7.570	10.219	14.974	21.227	3	3	3	3	0.028	0.057	0.108	0.264	180	250	300	330
16	60	75	YES	7.002	9.300	13.899	21.593	3	4	4	4	0.017	0.043	0.103	0.348	220	290	340	410
17	60	25	YES	7.886	11.314	18.400	30.394	4	4	4	4	0.033	0.081	0.202	0.533	220	290	340	410
18	70	75	NO	7.389	10.450	15.792	23.218	4	4	4	4	0.027	0.062	0.122	0.336	200	270	320	370
19	80	50	NO	6.124	9.146	12.414	19.601	3	4	4	3	0.016	0.041	0.075	0.187	190	260	310	340
20	70	50	NO	7.016	10.640	15.336	22.134	3	4	4	4	0.025	0.062	0.132	0.388	180	270	320	370

3.2. Analysis of Variance of the Responses

Results of ANOVA analysis are shown in Table 7. As shown from the reported *p*-values, all the models are statistically significant, with only two exceptions, NoP_15D and NoP_20D, that show *p*-values well above 0.05. Therefore, for the great majority of the responses it was possible to continue the analysis by evaluating the quality of the fitting and, eventually, the significant factors. Regarding the quality of the fitting, the great majority of the responses showed fairly good values as they were around 0.5 or over. Nevertheless, it is important to note that responses such as Height_30D, NoP_30D and LAI_15D, showed too low R^2 values (around 0.3) to consider associated models representative of the data, and, for this reason, these responses were not further considered in the analysis. Observing the data (Table 6), this result is due to the fact that for Height and NoP, increasing the time of observation, had well enough constant data registered at the highest value possible for the plant, independently of the growing conditions. At the same time, LAI observations for different experiments at too few days resulted in data too similar to each other, due to the fact that the leaf area observed was generally restrained. Taking into consideration the evolution of the same property over time, models associated with LAI and Water responses better explained variations over time, as R^2 increased with the number of days of observation. In contrast, among responses related to Height, the highest R^2 value was for the observation at 20 days. The predicted R^2 values (Table 7) show the same trends as R^2, according to its definition [36].

Table 7. Results of ANOVA analysis**.**

Response		*p*-Value Model	R^2	Pred R^2	Significant Factors
Height	15D	0.0044	0.47	0.26	HR:DB White
	20D	0.0002	0.80	0.73	Distance HR:DB White HR:DB-White $(HR:DB)^2$
	25D	0.0012	0.55	0.36	Distance HR:DB
	30D	0.0096	0.32	0.14	
NoP	15D	0.2793			
	20D	0.3467			
	25D	0.0178	0.46	0.17	Distance White Distance-White
	30D	0.0137	0.29	0.14	

LAI	15D	0.0185	0.27	0.12	
	20D	0.031	0.49	0.30	Distance HR:DB
	25D	0.003	0.61	0.48	Distance HR:DB
	30D	<0.0001	0.67	0.58	Distance
Water	15D	0.0005	0.50	0.41	Distance
	20D	<0.0001	0.62	0.53	Distance
	25D	<0.0001	0.62	0.53	Distance
	30D	<0.0001	0.87	0.83	Distance

3.3. Quantitative Calculation of the Mathematical Models

Table 7 also lists significant factors for the evaluation of responses, while Figures 3–6 show graphically the quantitative estimation of coefficients related to each significant factor (individually or interacting) for responses with a statistically reliable fitting of the models.

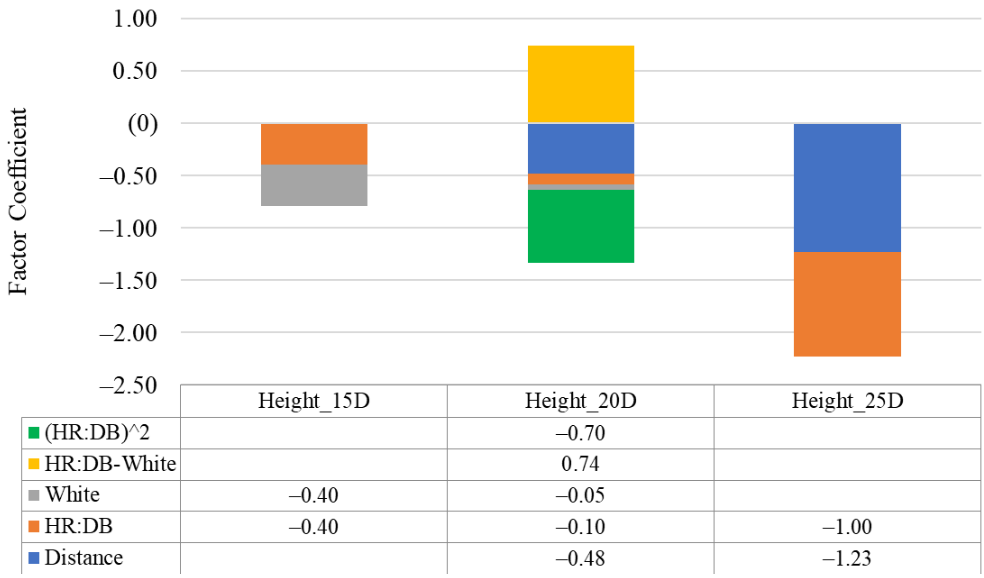


Figure 3. Coefficients of the significant factors for statistically reliable models associated to Height. The associated error is equal to 0.05%.

Distance was always relevant individually or interacting with other factors, except for Height_15D (Table 7), and always with a negative value (Figures 3–6), meaning that all the responses increased when basil plants were closer to LED modules (60 cm). This means that having plants closer to LED modules helps basil growth. On the one hand, the reduction of the plant-light distance is a positive effect for all the responses, as the objective is to maximize them, except for water usage which should be minimized (Table 5), also allowing growth in less volume. On the other hand, the lower height should be carefully considered, and a tradeoff is needed. The smaller the distance, the higher the illumination uniformity (thanks to the lens used to reduce the emitting cone of the LEDs), but the smaller the area illuminated by the LED modules (i.e., reduced number of plants potentially growing under the same module), the higher the risk of damage to the canopy of plants due to high heat absorption. In this context, the minimum distance of 60 cm can be evaluated as a good compromise to maximize the irradiance area without risk of damage to the canopy of plants, guaranteeing in first approximation the same irradiance for all the nine pots used in the same test.

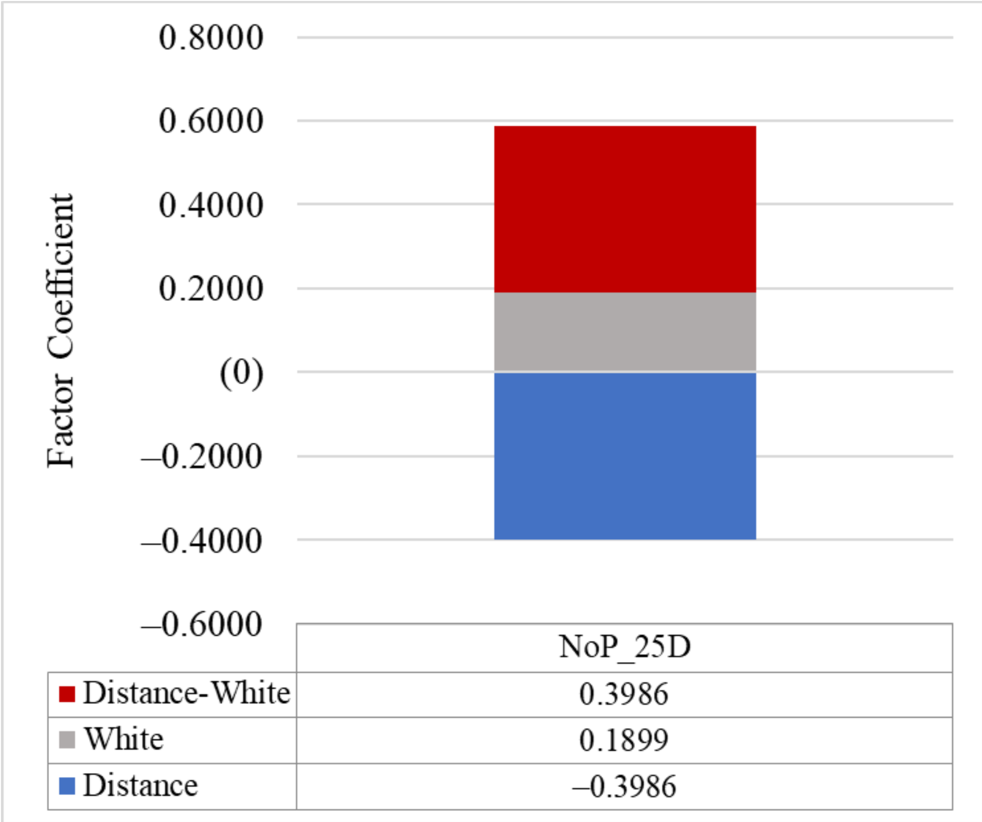


Figure 4. Coefficients of the significant factors for statistically reliable models associated to NoP. The associated error is equal to 0.05%.

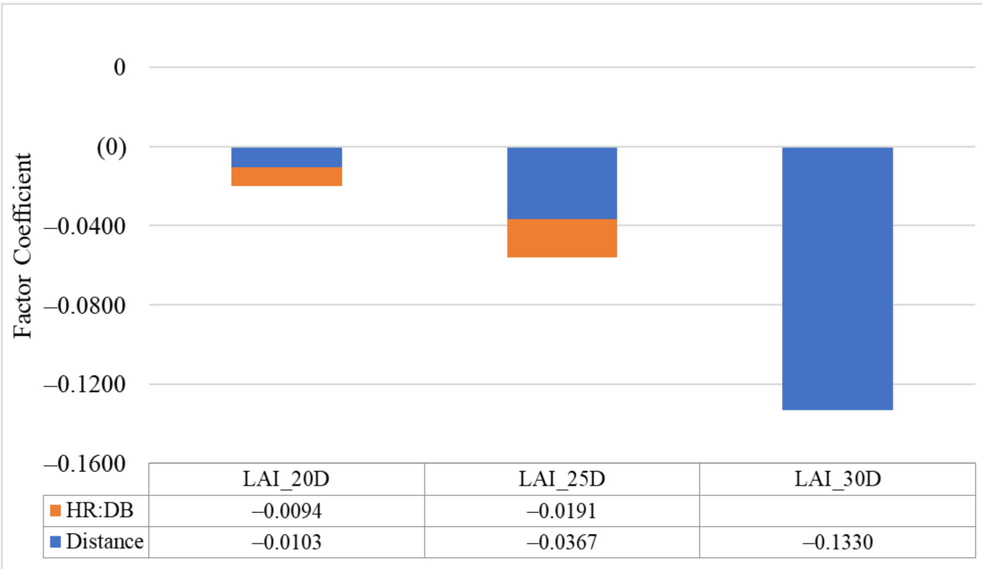


Figure 5. Coefficients of the significant factors for statistically reliable models associated to LAI. The associated error is equal to 0.05%.

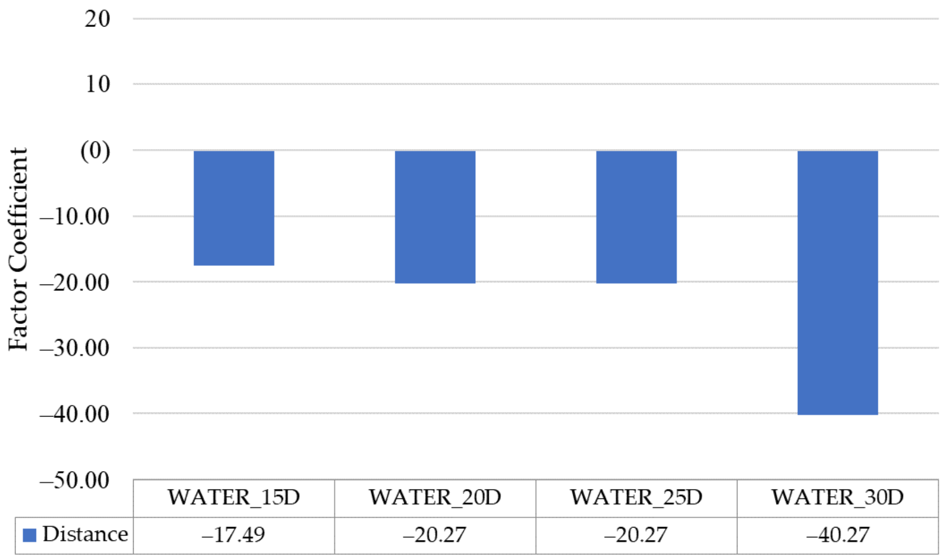


Figure 6. Coefficients of the significant factors for statistically reliable models associated to Water. The associated error is equal to 0.05%.

Moreover, Distance becomes more and more relevant when increasing the number of days of observation, as its coefficients (in absolute values) increase (for example from −17.49 to −40.27 for water-related responses), becoming the only significant factor for all the responses at 30 days. It is worth noting that the responses that were mainly affected by the factor Distance were the ones associated with water usage, accordingly with the fact that water usage is related to plant heating due to artificial light and, therefore, with light–plant distance (Figure 6).

The HR:DB ratio had an influence only on Height (Figure 3) and LAI (Figure 5) responses, and only for the first 25 days of growth. Regarding this factor, when its effect is individually, a negative effect is always reported; therefore, low values of this factor (25%) will generally promote Height and LAI. On the other hand, a positive synergic effect is observed when it is coupled with the employment of White LEDs to promote Height_20D and NoP_25D (Figures 3 and 4). This important achievement demonstrates how different LED light combinations differently affect and promote basil properties at different growth stages.

In strong similarity, the addition of White LEDs that was statistically relevant for the responses Height_15D, Height_20D (Figure 3) and NoP_25D (Figure 4), had a negative individual effect on the responses related to Height, but in synergy with HR:DB (Figure 3), it promoted the development of basil height. Again, for the response NoP_25D, an already positive individual effect of the addition of White LEDs was further remarked by the synergy with Distance (Figure 4).

From the calculated coefficients reported in Figures 3–6, mathematical models were derived in terms of equations and graphs. Equations were derived by applying Equation (1) and are reported in Table 8, whereas from Figures 7–10, contour plot graphs, representative of the statistically reliable mathematical models, better highlight the achieved results regarding basil growth parameters, according to the explanation given in Section 2.4.

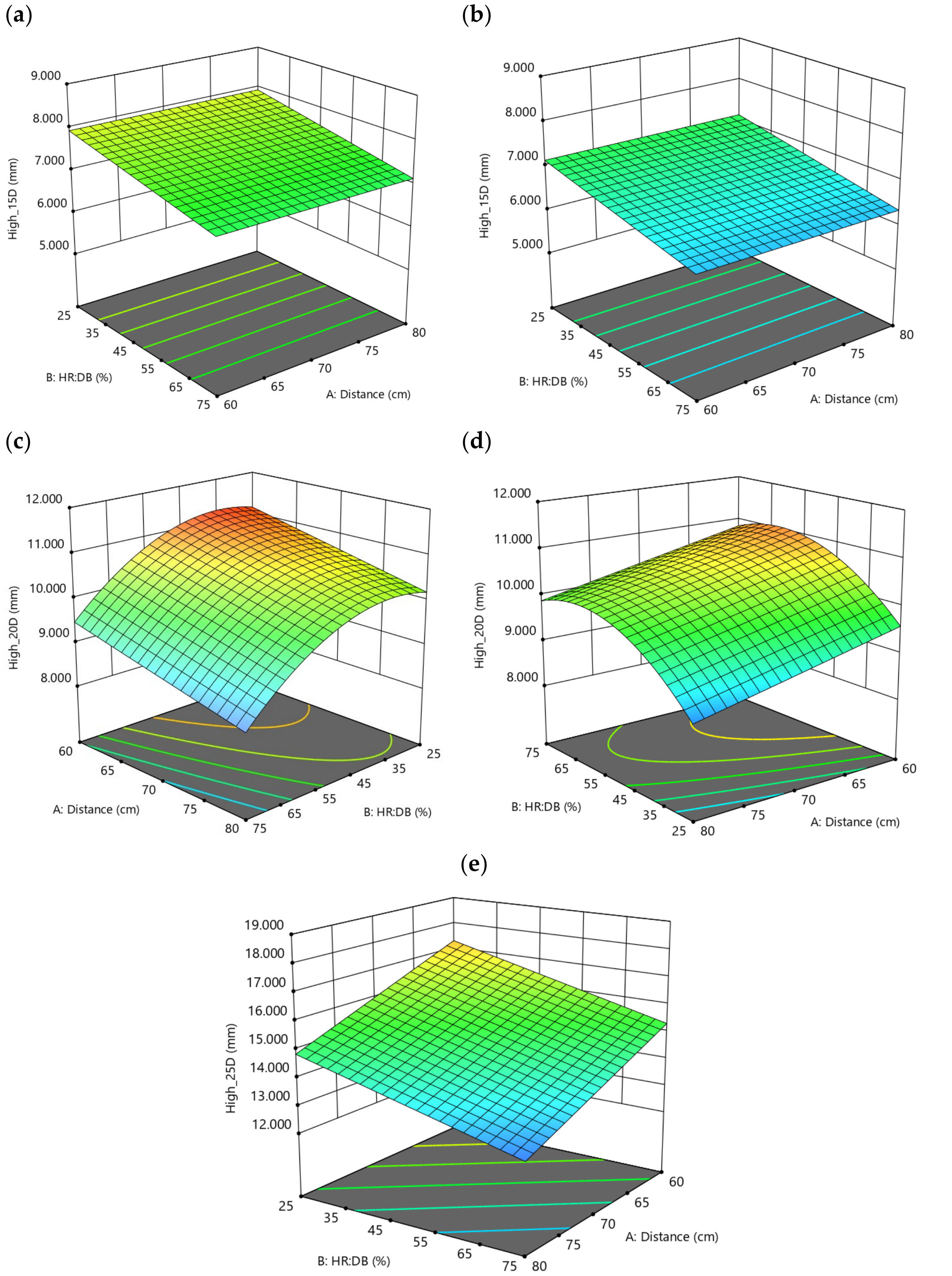
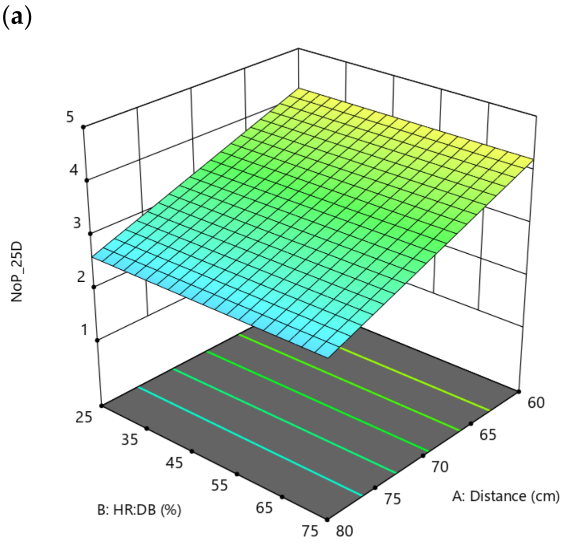


Figure 7. Contour plots of the models related to the property Height: (**a**) Height_15D with White LEDs; (**b**) Height_15D without White LEDs; (**c**) Height_20D with White LEDs; (**d**) Height_20D without White LEDs; (**e**) Height_25D.

Table 8. Equations of the statistically reliable mathematical models calculated in terms of actual components.

Height	15_D	=8.33241 − 0.0158963 * B (If WHITE = YES) =7.53981 − 0.0158963 * B (If WHITE = NO)
	20_D	=12.8146 − 0.0483811 * A + 0.0777525 * B − 0.00111477 * B^2 (If WHITE = YES) =9.75719 − 0.0483811 * A + 0.136848 * B − 0.00111477 * B^2 (If WHITE = NO)
	25_D	=25.6975 − 0.1231 * A − 0.0400614 * B
	30_D	/
LAI	15_D	/
	20_D	=0.155884 − 0.000991378 * A − 0.00103501 * B + 6.12105 × 10^{-6} * AB
	25_D	=0.530544 − 0.0049938 * A − 0.0040929 * B + 4.14395 × 10^{-5} * AB
	30_D	=1.45793 − 0.0142164 * A − 0.00694369 * B + 5.99759 × 10^{-5} * AB
NoP	15_D	/
	20_D	/
	25_D	=9 − 0.0797101 * A (If WHITE = YES) =3.8 − 5.55112 × 10^{-17} * A (If WHITE = NO)
	30_D	/
Water	15_D	=325.058 − 1.74903 * A
	20_D	=418.378 − 2.02703 * A
	25_D	=468.378 − 2.02703 * A
	30_D	=658.378 − 4.02703 * A

A = Distance; B = HR:DB.



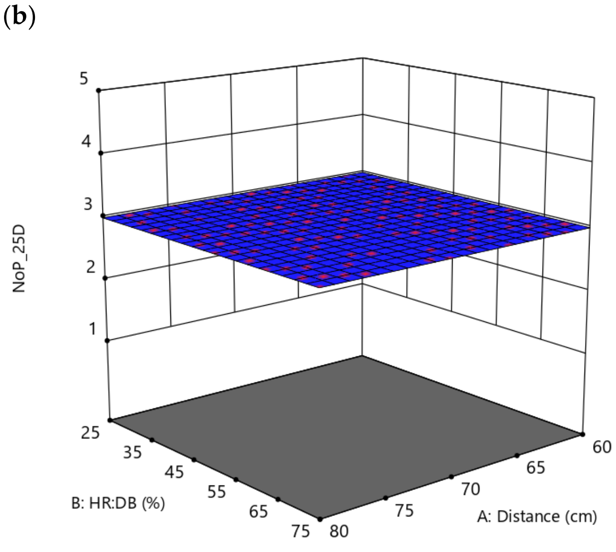


Figure 8. Contour plots of the models related to the property NoP: (**a**) NoP_25D with White LEDs; (**b**) NoP_25D without White LEDs.

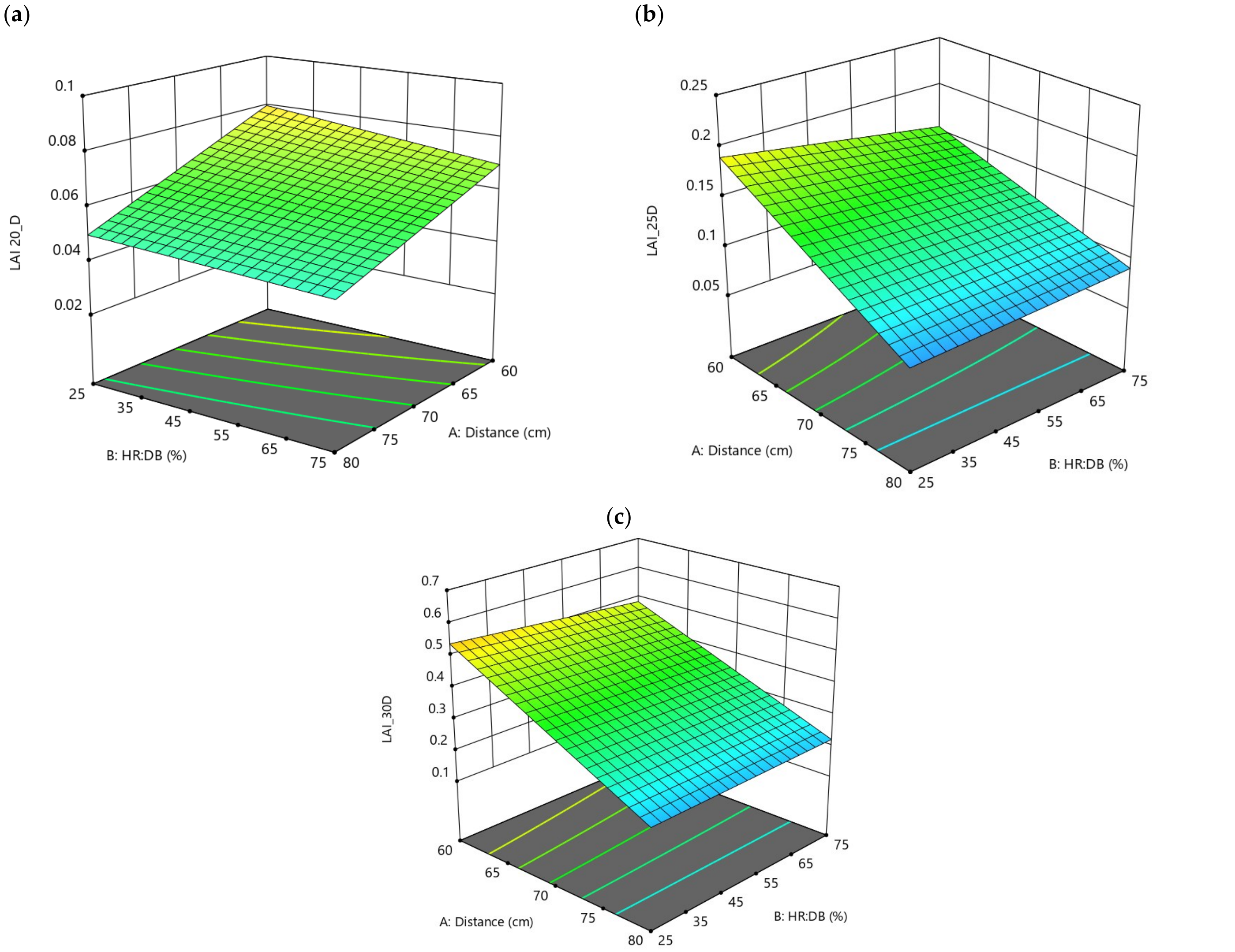
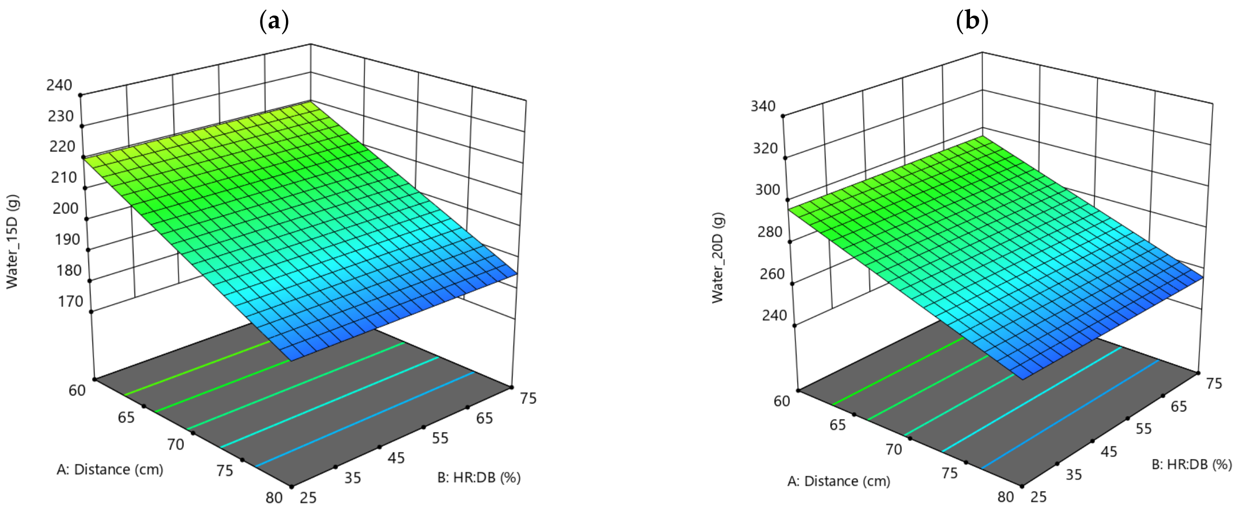


Figure 9. Contour plots of the models related to the property LAI: (**a**) LAI_20D; (**b**) LAI_25D; (**c**) LAI_30D.



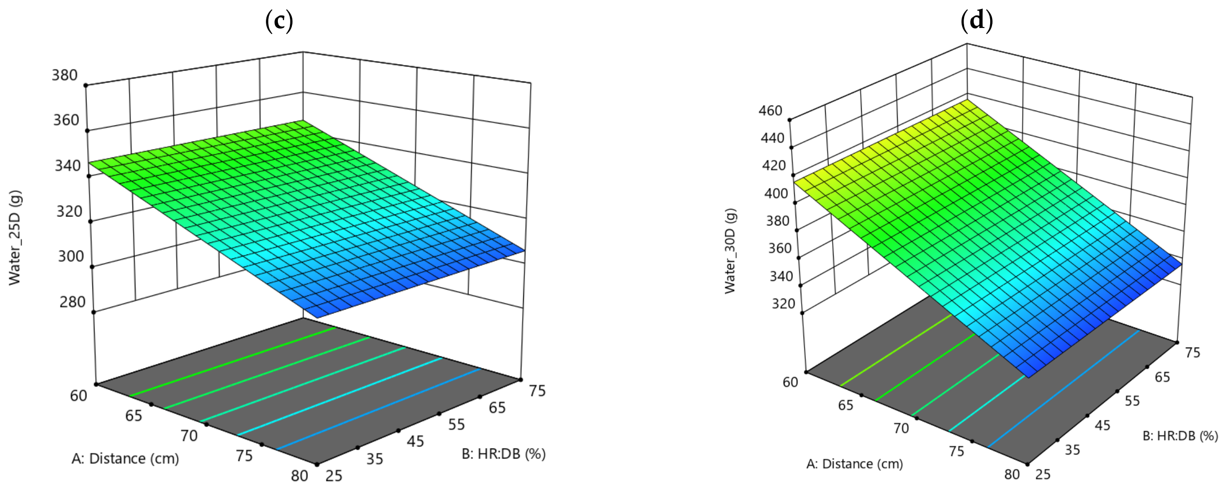


Figure 10. Contour plots of the models related to the property Water: (**a**) Water_15D; (**b**) Water_20D; (**c**) Water_25D; (**d**) Water_30D.

Regarding Height, all the models are presented in Figure 7, where it is possible to clearly see that the best conditions to further enhance this property in all the growth stages are: Distance equal to 60 cm, presence of White LEDs and ratio HR:DB equal to 25%. In fact, in the first growth phase (Figure 7a,b) the presence of White LEDs as well as a restrained HR:DB ratio would enhance this response. In the second growth phase (20D), this trend is constant, but a restrained Distance (60 cm) would further enhance the Height at this stage of growth (Figure 7c,d). Finally, in the third stage (25D), the addition of White LEDs is no longer statistically reliable, and only a synergy between a restrained Distance and the lowest HR:DB ratio would further maximize the basil height (Figure 7e). From this it can be derived that White LEDs are absolutely necessary only during the first 20 days, whereas after this time their presence is no longer statistically relevant.

Considering NoP, the only reliable model is the one calculated after 25 days of observation, and it is represented in Figure 8. From this model, again the better conditions to maximize this property (Figure 8a) involve the presence of White LEDs and a restrained distance among LEDs and light (equal to 60 cm), while the ratio HR:DB can be chosen without affecting this property.

Regarding LAI (Figure 9), considerations quite similar to Height can be made, as the best condition to enhance this property during all the growth phases are driven by restrained Distance (equal to 60 cm) and ratio HR:DB (equal to 25%). In contrast to Height, LAI is not dependent on the presence of White LEDs and the ratio HR:DB plays a role only until 25 days of observation (Figure 9a,b), since after this time each combination of HR:DB tested was equivalent to another as shown in Figure 9c

Finally, considering water consumption, the models are reported in Figure 10. From these Figures, it is clear that the only parameter that affects this property along all the time of observation is *Distance*, as already seen in Table 6 and moving from the 15D (Figure 10a) to 30D (Figure 10d) observations its importance increases. Therefore, to minimize water consumption, with the aim to save such a relevant resource, the greatest Distance (80 cm) should be kept.

3.4. Mathematical Models Validation

Based on the observations made before, it is clear that it is not so simple to find a light recipe that can optimize at the same time all the basil properties at each growth phase. Therefore, the desirability function has also been calculated for each time period, according to the information provided in Section 2.4 and Table 5. Results regarding the desirability function are shown in Figure 11, where contour plots concerning the function have been provided. In these graphs, similar to the other contour plots, it is possible to immediately identify the area of optimum (in yellow) that would suggest the best light recipe for each time period. For the first time period (15D) the best condition is due to the interaction of a restrained HR:DB ratio (25%) with the lowest distance (60 cm) and the presence of White LEDs (Figure 11a). Considering the information reported in Table 1, the overall HR:DB ratio for this best condition is equal to 44%. It is the same for the second time period (20D), even if in this case a larger yellowish area can be observed (Figure 11b). In the third time period (25D), a difference from the previous ones arises as the White LEDs should be avoided (Figure 11c). Finally, the fourth period (30D) is the one less affected by the factors investigated here since only distance plays an effective role, meaning that all the LEDs can be shut down without damaging basil growth (Figure 11d).

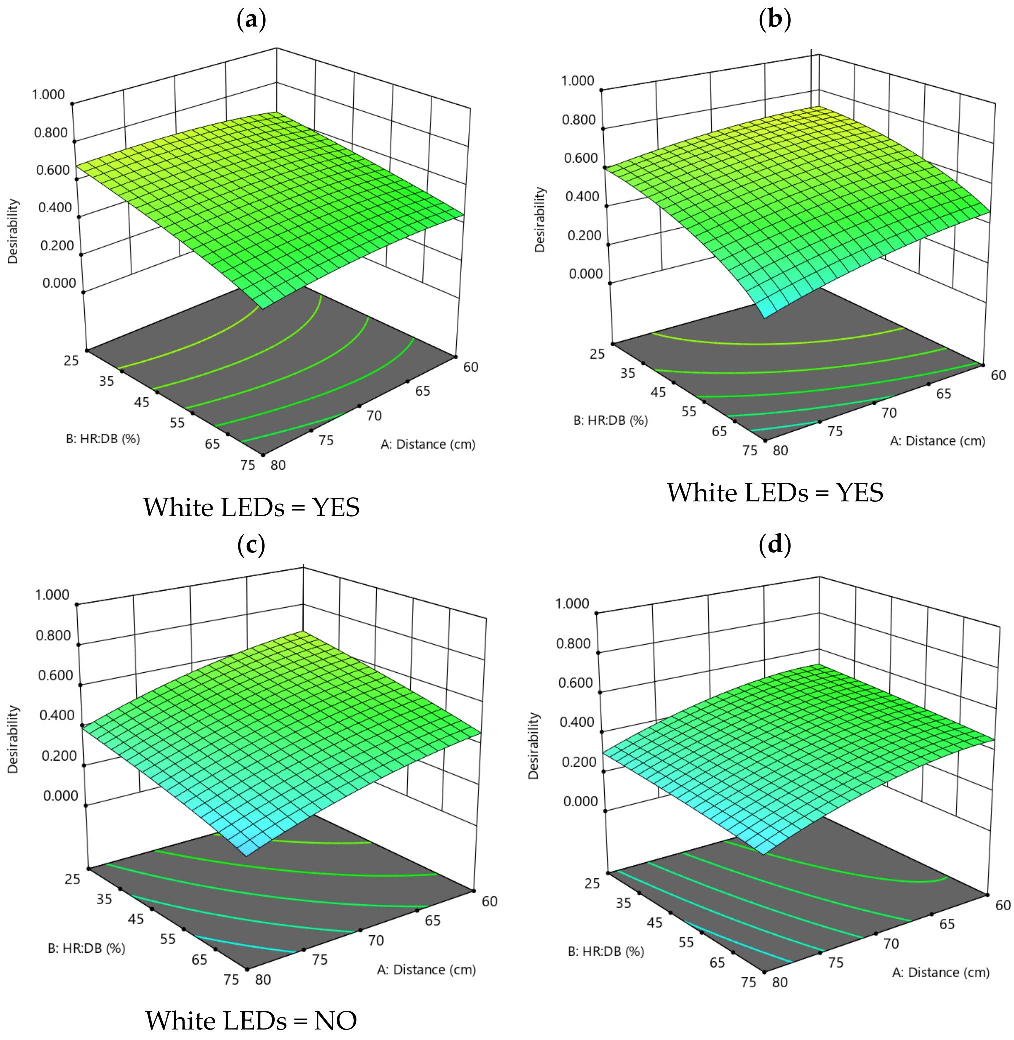


Figure 11. Contour plots of the desirability functions related to different periods: (**a**) 15D; (**b**) 20D; (**c**) 25D; (**d**) 30D.

The numerical results of this evaluation are reported in Table 9, as well as experimental data concerning the desirability function validation. In particular, to experimentally validate the guidelines obtained from the results' analysis, an additional growth run was carried out having these main features: (i) light source—plants distance fixed at 60 cm; (ii) fixed photoperiod of 16h/day; and (iii) light recipe varying during the growth period in accordance with the results from Figures 7–10 and Table 8. The employed light recipes are summarized in Table 10.

Table 9. Summary of the best solutions found by the desirability function and its experimental validation. Data regarding water are not present due too high % errors on the predicted values.

		15_D	20_D	25_D	30_D
Optimized Light	Distance (cm)	60	60	60	60
	HR:DB (%)	25	25	25	-- *
	White	YES	YES	NO	-- *
Predicted	Height (cm)	7.93 ± 1.20	11.12 ± 2.12	17.31 ± 2. 32	24.34 ± 2.23
	LAI	0.08 ± 0.01	0.18 ± 0.01	0.28 ± 0.01	0.50 ± 0.21
	NoP	3.2 ± 0.5	3.7 ± 0.6	4.2 ± 0.6	4.1 ± 0.5
Validation	Height (cm)	9.37 ± 1.02	15.00 ± 2.54	20.16 ± 2.52	26.49 ± 2.50
	LAI	0.09 ± 0.02	0.18 ± 0.04	0.23 ± 0.05	0.30 ± 0.07
	NoP	3.9 ± 0.7	4.4 ± 1.0	4.3 ± 1.0	4.3 ± 1.00

* The symbol—means that there is not an optimal condition, but each solution fits the model.

Table 10. Light recipe used in the validation test.

Growth Period [Days]	Combined Module's Code [1]	PPF HR [μmol/s]	PPF DB [μmol/s]	PPF WW [μmol/s]	Total PPF_{LED} [μmol/s]	$PPF_{effective}$ [2] [μmol/s]	%PPF HR	%PPF DB	%PPF White
0–20	8HR 10DB 6WW	16.11	22.62	8.84	47.57	26.93	33.87	47.56	18.58
21–25	6HR 18DB	36.25	13.57	0.00	49.82	28.21	47.31	33.82	18.87
26–30	18HR 6DB	12.08	40.72	0.00	52.80	29.90	61.19	19.64	19.18

[1] Module's coding: <# HR LEDs>HR: <# DB LEDs>DB: <# WW LEDs>WW. [2] estimted by considering the LED's lens efficiency and ratio between the growth area and the illuminated one as described in Section 2.1.

3.5. Discussion

It is worth noting that the results analysis of the experimental plan suggests that during the last days of the considered growth cycle (days 26–30), the light recipe had no substantial effects on the plant's parameters of interest. Therefore, with a green economy perspective, the one having the lower power consumption can be chosen.

Summarizing, it clearly appears that it is important to consider the presence of White LEDs to maximize growth properties, in combination with HR:DB ratio, only at the beginning of the growth period (15, 20 and 25 days of observation).

This result is in agreement with the literature and concerns not only basil, but also other vegetables. Customized light recipes with different colors of the light spectrum and different mutual ratio and interactions are also important for plant development [38,39]. For example, it has been found in the literature for radish microgreens, cabbage, broccoli and basil [27,20], and further confirmed for basil in this study (Table 10), that the increase in the percentage of blue light after a certain amount of time had a negative effect on plant growth and yield, so best growth performances can be obtained by limiting it. Nevertheless, blue light is capable of reducing edema and improving basil compactness; therefore, a small quota of this light should be kept [20]. In addition, the importance of White LED light on the growth performance of basil has been clearly assessed, improving the results already reported in our related work [26] and in agreement with previous literature [27–29,40]. Nevertheless, it must be noted that the proportion of reddish light to white light should always be favorable to reddish light, as it is more capable of increasing canopy yield by improving photosynthesis, as previously reported in the literature [28,40]. On the other hand, towards the end of the growth (30 days of observation), Distance, or light intensity, was the only important factor. This means that, at 30 days, light intensity is the only factor which must be taken into account to enhance basil growth, and each ratio of HR:DB or the presence or absence of white LEDs, can be chosen without affecting basil growth. Since it is important to obtain a given light intensity, the selection of light recipes could be based only on one goal, i.e., the lower energy consumption. This approach is similar to the one studied by Pennisi et al. which considered different light intensities with a given light recipe [23]. As the relevance of distance or light intensity increases with time, it is important to choose the right distance between plants and LED modules, depending on which parameter is necessary to promote. In fact, a wise choice could be to bring the plant canopy closer to the modules, avoiding damages due to too strong light intensity. An initial large distance would mean an increased use of space, which is a problem for an indoor farm, while a short distance would mean that the canopy of growing plants would come too close to the LED modules. Regarding the canopy morphological characteristics, this result is in agreement with the one obtained by Modarelli et al. about red lettuce; higher light intensities promoted several morphological adaptations, increasing leaf thickness and stomatal density that resulted in a more compact canopy [41]. This fact could force the reduction of power given to modules to avoid damages to the plants or excessive water consumption, hindering the optimal exploitation of LEDs. In addition, it must be noted that, according to previous literature, the employment of a well-tailored LED light recipe is capable of reducing the overall electrical energy consumption of a vertical farm, with respect to conventional artificial light (HPS lamp) by also improving the yield [42,43], with additional cost savings of about 25% if load shifting techniques are applied [42,43]. Comparing these results with similar studies, it is interesting to note that different growth environments could strongly change the optimal ratio of LED lights. In fact, according to Lin et al., for hydroponic cultivation of green basil the great majority of Red LEDs should be used to enhance basil growth [44]. As suggested from Sipos et al., the basil growing conditions should be optimized for each specific controlled environment, and for this matter statistical methods could be very helpful [21]. Regarding validation, the data reported in Table 9 compares the growth properties predicted from the mathematical models with collected experimental data obtained from further and specific experimental tests, in which the obtained best light recipes have been employed. These data confirm the good predictive power of the models and the fact that the calculated light recipes are capable of promoting the basil properties. In fact, comparing Table 9 with Table 6 , it appears clearly that in the validation dataset the highest parameters in terms of Highness, Number of Plants and LAI have been obtained for all the considered periods of growth. Nevertheless, it must be noted that, among the limits of the statistical approach, the results here obtained are valid for basil growth in the conditions established in this work, e.g., keeping the same soil and the same type of LEDs. Therefore, further work must be done to verify the validity of the results for other plants or in different soil conditions, for example.

4. Conclusions

This work studied, through Design of Experiment (DoE) methodology, the optimization of basil growth in an indoor controlled environment using only LEDs as the light source. Considering five-day time steps of growth, the influence of three different factors on basil was considered for the first 30 days of growth, namely: light intensity expressed as distance between LEDs and plants canopy, HR:DB ratio, and presence of White LEDs light. Responses, in terms of growing properties such as plant height, number of plants, LAI, and the amount of water used, were measured and mathematical model calculation was performed, at 15, 20, 25, and 30 days after sowing. The models generated through DoE found that in the first days of growth (15–25 days) the role of light recipe was more important than white LEDs, and a precise ratio of Hyper Red and Deep Blue LEDs equal to 44% must be employed to maximize the growing parameters. The role of distance, or light intensity, became more important with time, and it was the only significant factor that determined the four responses of basil plants at 30 days, and it must be kept restrained, thereafter equal to 60 cm, to maximize the majority of the investigated properties. For future perspective, a comparison between other types of plants (green leaf or not) can be proposed regarding the morphological growth of the plant to understand if a constant behavior subsists. In addition, the nutraceutical composition could be evaluated as a further response to also optimize basil employment in function of its use (e.g., for food or for cosmetics applications).

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