



Optimizing *in vitro* growth of basil using LED lights

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ABSTRACT: *Tissue culture is an important technique for assessing the influence of light on plant growth. This study evaluated the in vitro cultivation of Ocimum basilicum cultivars under different light sources. First, an experiment was conducted to evaluate the effect of fluorescent and LED (Light Emitting Diode) light sources (yellow, blue, green, and red) on five basil cultivars (Cinnamon, Grecco a Palla, Italian Large, Limoncino, and Maria Bonita), followed by, another experiment, with two LED lights (Growlux and Blue). In vitro basil plants did not exhibit good growth under yellow lamps. The size of the roots of Cinnamon, Grecco a Palla and Limoncino cultivars under the two light conditions were similar. Moreover, the luminous condition of the Growlux lamps generated a greater quantity of leaves and fresh, and dry weight than blue LED lamps for Cinnamon, Grecco a Palla and Limoncino cultivars. Thus, it was concluded that the Growlux lamps promoted a greater amount of basil biomass.*

Key words: *Ocimum basilicum, micropropagation, fluorescent, cultivars.*

Otimização crescimento *in vitro* de manjeriço usando lâmpadas LED

RESUMO: *A cultura de tecidos é uma técnica importante para avaliar a influência da luz no desenvolvimento das plantas. O objetivo deste trabalho foi avaliar o cultivo in vitro de cultivares de Ocimum basilicum sob diferentes fontes de luz. Primeiramente, foi avaliado o efeito de fontes de luz fluorescentes e LEDs (Diodo emissores de luz amarela, azul, verde e vermelha) em cinco cultivares de manjeriço (Cinnamon, Grecco a Palla, Italian Large, Limoncino e Maria Bonita); e posteriormente o efeito de duas lâmpadas LED (Growlux e Azul). As lâmpadas amarelas não proporcionaram um bom desenvolvimento das plantas de manjeriço in vitro. Verificou-se que o tamanho das raízes e massa fresca nas cultivares Cinnamon, Grecco a Palla e Limoncino nas duas condições luminosas foram semelhantes. A condição luminosa das lâmpadas Growlux promoveu maior número de folhas, massa fresca e seca quando comparada com lâmpadas LED azuis nas cultivares Cinnamon, Grecco a Palla e Limoncino. Assim, concluiu-se que as lâmpadas Growlux promovem uma maior quantidade de biomassa de manjeriço.*

Palavras-chave: *Ocimum basilicum, micropropagação, fluorescente, cultivares.*

INTRODUCTION

Light is a very crucial factor in plant cultivation because it transmits radiant energy and is responsible for exciting the photosynthetic apparatus, thereby allowing its maintenance. In the case of *in vitro* plant culture, light factors can affect an aspect of plant growth and can be strategically used for their rapid and large-scale propagation (PAWŁOWSKA et al., 2018). The spectral qualities of light contain environmental parameters used by plants as clues to modify their biology to adapt and survive. One type of lamp that has been used in growth rooms is LEDs (Light Emitting Diode) because of their benefits

including monochromatic spectrum, less heat, and consequently less expense for controlling the room temperature by air conditioning. In addition, the lower heat output allows the lamps to be positioned closer to the plants allowing for better lighting intensity (EAVES & EAVES, 2018; BATISTA et al., 2018).

LED-based lamps, with their highly definable spectral properties, offer great prospects for use in the *in vitro* production. The effects of spectral qualities of light on plant physiology are not yet fully understood, and previous studies have analyzed short-term of effects or the use of monochromatic light treatments in closed growth chambers (JESEN et al., 2018).

The LED lamp is an electronic component that does not use a reactor and generates light with low energy consumption, as it requires less power to generate the same luminous flux as a fluorescent lamp (lumens). When maintained at favorable temperatures, LEDs have an operating life of 50,000 h or more, which is at least twice as long as conventional fluorescent or high-pressure sodium lamps (KOKSAL et al., 2015).

Previous studies reveal positive results related to light manipulation through different types of LEDs and associated growth in different groups of plants like sugarcane (FERREIRA et al., 2016), banana cultivars (ROCHA et al., 2017), gerbera (PAWŁOWSKA et al., 2018) and alpinia cultivars (PINHEIRO et al., 2019). The results generally emphasize the relationship between different responses according to the cultivars and the type of LED used.

According to PARADISO & PROIETTI (2021), the optimal light spectrum and intensity required by many crops are still unknown. This information is important for optimizing yield, product quality, food security, and profitability (RAHMAN et al., 2021).

The species *Ocimum basilicum* is an aromatic and medicinal plant popularly known as basil. It belongs to the botanical family Lamiaceae, is annual or biennial, and can grow in open-fields as well as under greenhouse conditions, in a wide range of climatic conditions. The flowers are small and whitish or purple, gathered in short terminal clusters, and the seeds are small, black, and orthodox (AL ABBASY et al., 2015).

Basil has a pronounced flavor and aroma, and thus, it is much appreciated for fresh consumption and as a culinary spice. The demand for basil has recently increased owing to its strong antioxidant capacity, correlated with a reduced risk of certain types of cancer and degenerative diseases (SGHERRI et al., 2010; PETROPOULOS et al., 2020).

The use of LED combinations with the color blue (B):red (R):white (W) ratio has been reported in the indoor cultivation of sweet basil. SONG et al. (2020) related the optimal ratio to B0:R5:W5 ($128 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), to promote the growth of sweet basil; however, NAZNIN et al. (2019) recommended B1:R9:W0 ($200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and PENNISI et al. (2019a) recommended B3:R7:W0 ($215 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Other colors and ratios are also present in the previous studies, such as DOU et al. (2019), who recommended white light with a low green proportion (10%).

Despite these reports, the behavior of basil under optimal light conditions for *in vitro* cultivation is still not clear. Therefore, the present study evaluated the biometric and physiological characteristics in the *in vitro* cultivation of basil cultivars under different light source.

MATERIALS AND METHODS

The first study was carried out using basil (*Ocimum basilicum*) seeds from Cinnamon, Grecco a Palla, Italian Large Leaf, Limoncino and Maria Bonita cultivars provided by Richters Herbs.

The seeds were disinfected in 70% alcohol for 1 min and then in 30% sodium hypochlorite solution for 20 min. Afterward, they were washed three times with distilled water in a laminar flow chamber and autoclaved.

The culture medium comprised $\frac{1}{2}$ of MS salts and vitamins (MURASHIGE & SKOOG, 1962) and 3% sucrose plus 1.8 g L^{-1} of phytagel. The pH was adjusted to 5.7 before autoclaving at $121 \text{ }^\circ\text{C}$ and 1.2 atm for 20 min. Subsequently, the seeds were inoculated in transparent glass vials of 100mL containing 30 mL of culture medium, sealed with plastic lids and placed in a growth room for seedling development for 60 days. The growth room was maintained under a photoperiod of 16-h per day at a temperature of $25 \pm 2 \text{ }^\circ\text{C}$, with a luminous intensity of $25 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$, supplied by white fluorescent lamps.

After 60 days, the explant nodal segments of 2 cm were excised and tested under MS medium underwith the following light conditions: white fluorescent and yellow, blue, green and red LEDs (Figure 1). The lamps used on the experiments was Ourolux® brand, 9 Watts. A Styrofoam was placed covering the shelves to avoid interference between the lamps. The experimental design used was randomized blocks with five treatments and four repetitions. Each experimental plot was composed by ten vials, each containing five nodal segments.

The homogeneity of the residual variances was verified using the ratio between the largest and the smallest mean square of residue, considering the value seven as the limit for the validation of the analysis. At 60 days the inoculation of nodal segments, the following characteristics were evaluated: number of leaves, number of shoots, length of shoot (cm), and fresh and dry weight (g) of seedlings developed in addition to the Spad index obtained through a chlorophyll meter. The Spad - Soil Plant Analysis Development SPAD-502 Index (MINOLTA, 1989) is measured using a device that generates dimensionless quantities related to chlorophyll content.



The second study was installed in a similar way to the previous one; the nodal basil segments of Cinnamon, Grecco a Palla, and Limoncino cultivars were studied because they were the ones that best adapted to *in vitro* cultivation in the previous study. The flasks were placed in a growth room under two luminous blue LED lamps (Ourolux®, 9 Watts) and a Growlux LED (Gaungji®, 9 Watts).

The experimental design used was randomized blocks with three treatments and six repetitions. Each experimental plot was composed of five flasks, each containing five nodal segments.

At 60 days after the inoculation of nodal segments, the following characteristics were evaluated: number of leaves, number of shoots, shoot length (cm), and fresh and dry weight (g) of seedlings developed in addition to the Spad index.

The data obtained in all experiments were subjected to variance analysis and the assumptions of normality of residues, homogeneity of variances and additivity of blocks were tested and met, via the

Shapiro–Wilk, Levene and Tukey tests at $\alpha = 0.01$ respectively using the statistical program RStudio (R CORE, 2019). The characteristic number of leaves and the Spad index of first experiment (5 cultivars x 5 lamps) was transformed by (\sqrt{X}), for which real values were tabulated.

A combination analysis of the independent experiments was also conducted using the Genes statistical program (CRUZ, 2006) with subsequent comparison using the Tukey averages test at 5% probability.

RESULTS AND DISCUSSION

Through the analysis of variance of the five light source it was observed that there were no statistical correlations between the variation factors, but most of the characteristics evaluated in the *in vitro* basil cultivars differed from each other, except for the characteristic number of shoots (Table 1). Besides, it is possible to verify that the lamps tested in the *in*

Table 1 - Number of leaves (LEA), number of shoots (NSH), shoot length (LSH, cm), fresh weight (FWH, g), dry weight (DWH, g), Spad index (SPD) of seedlings of *Ocimum basilicum* cultivars under five light lamps.

Cultivars	LEA	NSH	LSH (cm)	FWH(g)	DWH(g)	SPD
Cinnamon	4.24 b	1.79 a	1.48 ab	0.12 c	0.016 b	5.20 b
Grecco a P.	8.65 a	1.81 a	1.84 ab	0.20 b	0.017 b	8.20 b
Italian L.L.	3.77 b	1.73 a	1.20 b	0.19 bc	0.018 b	13.72 a
Limoncino	5.08 b	1.76 a	2.69 a	0.25 b	0.025 b	14.80 a
Maria B.	6.12 b	2.05 a	1.29 ab	0.43 a	0.043 a	13.54 a
-----Lamps-----						
Yellow	5.04 bc	1.74 a	1.44 ab	0.22 ab	0.018 ab	10.32 b
Blue	9.38 a	1.70 a	2.74 a	0.26 ab	0.022 ab	13.84 a
Red	4.50 c	1.60 a	1.32 b	0.29 a	0.026 a	9.98 b
Green	6.40 ab	1.74 a	1.86 ab	0.16 b	0.016 b	10.62 b
Fluorescent	5.64 bc	2.04 a	1.7 ab	0.25 ab	0.022 ab	10.70 ab
CV	47.31	31.57	30.84	78.79	65.21	32.37

¹Averages followed by distinct letters in the column differ by Tukey test at 0.05 of significance.

in vitro cultivation of basil caused the differences in the number of leaves, shoot length, fresh, and dry weight, Spad index value (Table 1).

The number shoots produced by basil cultivars did not differ (Table 1). This characteristic is important to be analyzed because shoots produce and stand leaves that are the main producers of essential oil. One possibility for this result on amount of shoots is the number of days of conducting the experiment.

In all light environments tested in the first experiment, the cultivar Grecco a Palla produced a greater number of leaves than Maria Bonita, but the cultivar Maria Bonita compensated by producing a greater amount of fresh and dry mass and Spad index (Table 1).

The Spad indices of Italian Large Leaf, Limoncino e Maria Bonita cultivars were bigger than Cinnamon and Grecco a Pala. Regarding the type of lamp, the number of leaves of plants grown under blue LED was higher than that of plants grown under fluorescent light (Table 1). White fluorescent tubes are typically used in growth rooms of plant tissue culture laboratories; however, LED lamps consume less energy and cause less heating up of the growth room environment, considering both are of the same power.

The energy consumption in a tissue culture laboratory forms a large part of the production costs

therefore, blue lamps can be an alternative in the *in vitro* cultivation of basil for greater leaf production, which is one of the main raw materials for industry. Therefore, LED technology can contribute to the economic and increase biomass production, providing high secondary metabolite contents (CIOĆ et al., 2017).

Evidently, the type of light did not interfere with the number of shoots; however, it interfered with the length of these shoots (LSH). The blue LED light cause the *in vitro* plants to be larger than the red LED. This characteristic could be understood as a negative stagnation of the plants owing to the lower quality of light; however, these larger plants also had a higher number of leaves and Spad index (Table 1).

ARDELEAN et al. (2018) evaluated basil seedlings grown on *in vitro* conditions under blue, green, yellow and red LEDs found no effects of different LEDs on growth characteristics. The species *Salvia* sp., which is also an aromatic of the Lamiaceae family, when tested on controlled environments under different lamp colors showed greater lengths of the aerial part in cultivation in an environment of red LEDs and smaller lengths under 50% red and 50% blue light (WOLLAEGGER & RUNKLE, 2014), whereas HOSSEINI et al. (2019) suggested a ratio of 70% red + 30% blue light ($250 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$) for basil. This

reaffirmed the uniqueness of each species or cultivars and the need to test different types of light for each species before investing in a particular technology.

In addition, it was found that the dry and fresh weight produced by the basil plants were greater under red LEDs than under green ones; however, there are species of the Lamiaceae family where the combination of wavelengths in the range of green, red and blue were positive. (WOLLAEGER & RUNKLE, 2014).

Sweet basil presented higher crop yield and quality when illuminated with a combination of blue (B), red (R), and far-red (F) in comparison with only white LED illumination, emphasizing that sweet basil absorbs light strongly in the red and far-red, which corresponds to better plant performance (RAHMAN et al., 2021). According to PENNISI et al. (2019b) the red color is related to an increase in the water usage efficiency.

Using red light to grow basil in controlled environments was obtained positive effects of red light, with an increase in the number of basil leaves per plant, plant height, leaf size (length and width), and fresh and dry leaf weight (PATEL et al., 2018).

Evidently plants grown under red light exhibited lower leaf production, shoot length and Spad index. Although, its luminous flux is technically

the same as the other colored LEDs, it does not emit a sufficient quality for the growth of *in vitro* basil.

Through the analysis of variance of the Blue/ Growlux treatments, only the root length characteristic (LRO) did not differ (Table 2). The cultivars did not differ only in terms of the fresh and dry, while the tested luminous environments did not cause differences only in the Spad index value (Table 2).

The biometric characteristics assessed in the basil plants demonstrated that the number of leaves; and quantity of fresh weight and dry weight produced in the light condition of Growlux lamps were higher than those in cultivation with blue LEDs (Table 2). Growlux lamps are composed of beams of blue (460 nm) and red (660 nm) light, which is precisely the most favorable range for photosynthesis; and therefore, contributing to the overall optimal growth of plants. The Growlux type is manufactured for use in agriculture as well as in aquariums (SIPOS et al., 2020).

The length range existing in the Growlux lamps that are not present in the blue LEDs can also cause a larger diameter of plants and leaf area, both of which were not evaluated in this study and that would probably contribute to the advantage offered by this LED.

Table 2 - Number of leaves (LEA), number of shoots (NSH), shoot length (LSH, cm), root length (LRO, cm), fresh weight (FWH, g), dry weight (DWH, g) and Spad index (SPD) of seedlings of *Ocimum basilicum* cultivars under two LED lights.

Cultivars	LEA		NSH		LSH (cm)			
	Blue	Growlux	Blue	Growlux	Blue	Growlux		
Cinnamon	8.27 Ba	18.72 Aab	1.22 Aa	2.35 Ab	2.46 Aa	3.36 Ab		
G.a Palla	11.70 Ba	23.77 Aa	1.92 Ba	5.07 Aa	2.67 Aa	2.56 Ab		
Limoncino	8.20 Ba	13.90 Ab	1.52 Aa	2.12 Ab	1.79 Ba	4.88 Aa		
CV	23.36		32.37		22.29			
Cultivars	LRO (cm)		FWH (g)		DWH (g)		SPD	
	Blue	Growlux	Blue	Growlux	Blue	Growlux	Blue	Growlux
Cinnamon	2.57Aa	4.87Aa	0.326 B	0.956 A	0.108 B	0.220 A	17.14 a	17.01 ab
G.a Palla	3.44Aa	4.71Aa	0.370 B	1.144 A	0.080 B	0.260 A	8.84 b	11.75 b
Limoncino	3.27Aa	3.22Aa	0.396 B	1.105 A	0.119 B	0.212 A	17.29 a	20.81 a
CV	38.09		72.75		29.90		20.06	

¹Averages followed by distinct letters, upper case in the row and lowercase in the column, differ by Tukey test at 0.05 of significance.

The cultivar Grecco a Palla, when there was a difference, exhibited a greater amount of leaves and shoots, and a relatively shorter shoot length than Limoncino. This response pattern follows its trend when grown *ex vitro* and makes it an indicated option for ornamental use in pots (FRANÇA et al., 2017). Meanwhile, the cultivar Limoncino, when showing a difference, produced comparatively few leaves (Table 2).

The luminous condition of Growlux lamps enables shoots growth, but the latter were large which is also a characteristic verified *ex vitro* in this cultivar in another trial of this study. In addition, it was found that the cultivar Limoncino had a higher Spad index than the cultivar Grecco a Palla under both light conditions (Table 2).

The root length of the plants did not differ; this can be explained by the fact that the tested variation effects do not directly affect roots; they primarily interfere in the photosynthetic part of the plants because they have light-sensitive pigments (Table 2).

CONCLUSION

Under yellow, blue, red, green LED or fluorescent luminous environment Grecco a Palla cultivar produced a greater number of leaves than Maria Bonita and Maria Bonita cultivar produced a greater amount of fresh and dry weight and Spad index. The luminous condition of Growlux lamps produced a greater number of leaves, fresh and dry weight than blue LED lamps for the cultivars Cinnamon, Grecco a Palla and Limoncino *in vitro*.

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DECLARATION OF CONFLICT OF INTERESTS

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript.

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