# LED lighting for indoor cultivation of basil 

G. Pennisi ${ }^{1,2,3^{*}}$, A. Pistillo ${ }^{1}$, S. Nicola ${ }^{2}$, F. Orsini ${ }^{1}$, G. Gianquinto ${ }^{1}$, J.A. Fernández ${ }^{3}$<br>${ }^{1}$ DISTAL - Department of Agricultural and Food Sciences and Technologies, Alma Mater Studiorum Università di Bologna, Bologna. Italy<br>2DISAFA-VEGMAP, Department of Agricultural, Forest and Food Sciences, University of Turin, Turin. Italy<br>${ }^{3}$ Department of Agronomical Engineering, Escuela Técnica Superior de Ingeniería Agronómica, Universidad Politécnica de Cartagena, Cartagena. Spain<br>*giuseppina.pennisi@unibo.it


#### Abstract

Indoor cultivation systems are gaining importance worldwide, thanks to their greater efficiency in the use of resources (water, land and nutrients). The limiting factor for these systems is the illumination costs that are still high. In this context, LEDs (light emitting diodes) are gaining attention because of their ability to provide the required light spectra, and high electricity use efficiency. The goal of this study is to identify the role played by red:blue ( $R: B$ ) ratio on the resource use efficiency of indoor basil cultivation, linking the light physiological response to changes in yield and nutritional properties. Basil plants were cultivated in growth chamber under 5 different R:B ratio LED lighting regimens (respectively, $\mathrm{RB}_{0.5}, \mathrm{RB}_{1}, \mathrm{RB}_{2}, \mathrm{RB}_{3}$, and $\mathrm{RB}_{4}$ ), using fluorescent lamps as control ( $\mathrm{CK}_{1}$ ). For the six light treatments, a PPFD of $215 \mu \mathrm{~mol} \mathrm{~m}{ }^{-2} \mathrm{~s}^{-1}$ and a photoperiod of $16 / 8$ light/dark per day were provided. Greater biomass production was associated with LEDs lighting as compared with fluorescent lamp, with best performances observed using RB $\geq 2$. Adoption of $\mathbf{R B}_{2}$ and $\mathbf{R B}_{3}$ improved also the plant's capacity to transform resources, resulting in greatest water, land and energy use efficiency. Nutrient use efficiency was increased by using LED lights with a greater portion of blue light in the spectrum. Decreasing R:B ratio also increased leaf stomatal conductance. Plant grown under $\mathrm{RB}_{3}$ showed the best antioxidant properties in terms of flavonoid content and FRAP as compared to the other light treatments. From this study it can be concluded that a R:B ratio of $\mathbf{3}\left(\mathrm{RB}_{3}\right)$ provides optimal growing conditions for indoor cultivation of basil.


Keywords: Ocimum basilicum L.; water use efficiency (WUE); energy use efficiency (EUE); land surface use efficiency (SUE); nutrient use efficiency (NUE).

## 1. INTRODUCTION

One of the most important challenges for agriculture in the next 50 years will be the provision of food to feed ever larger cities with ever fewer resources. To face this scenario, new forms of agriculture, not dependent on arable land, are gaining increasing popularity [1]. This are indoor plant production system where environmental factors are controlled, minimizing the interaction with the external climate. They are known as Plant Factories with Artificial Lighting (PFALs) or Vertical Farms with Artificial Lighting (VFALs). Thanks to the possibility of developing cultivation on the vertical dimension by using multiple overlapping layers, the adoption of hydroponics systems, the opportunity to recover water loss for transpiration by plants, the recirculation of nutrient solution, indoor cultivation systems improve use efficiency of land, water, and nutrients [1-3]. On the other hand, the large use of energy for illumination, cooling, heating, and dehumidification, is limiting the diffusion of these systems [4].

Red and blue wavebands in the spectrum are considered the most important energy sources for photosynthetic $\mathrm{CO}_{2}$ assimilation [5], but the appropriate balance between red and blue
components in the light spectrum for indoor cultivation of leafy vegetables and herbs remains unclear.

The aim of this study is to identify the best red:blue (R:B) LEDs light ratio for biomass production, antioxidant capacity, and gas exchanges parameters of sweet basil indoor cultivation paying particular attention on how light quality can affect the use efficiency of the supplied resources (e.g., space, water, nutrients, energy).

## 2. MATERIALS AND METHODS

Four experiments were conducted in six separate compartments of a climate-controlled growth chamber (air temperature $24 \pm 2^{\circ} \mathrm{C}, 55-70 \%$ relative humidity and $450 \mathrm{ppm} \mathrm{CO}_{2}$ ) at the University of Bologna. Basil seeds (Ocimum basilicum cv. Superbo, Sais seeds) were germinated under fluorescent lamps (CK 1 , TL-D 90 De Luxe 58W, Philips) and, when plants reached the two true leaf stage, roots were washed and plantlets were transplanted into individual hydroponic system ( 100 plants $\mathrm{m}^{-2}$ ). Upon transplanting, six light treatments were applied: 5 different R:B ratio LED lighting regimens (respectively, $\mathrm{RB}_{0.5}, \mathrm{RB}_{1}, \mathrm{RB}_{2}, \mathrm{RB}_{3}$, and $\mathrm{RB}_{4}$, Flytech), and a fluorescent lamps as control ( $\mathrm{CK}_{1}$, see specs above). Plants were grown under artificial light only with a photosynthetic photon flux density (PPFD) of $215 \pm 5.5 \mu \mathrm{~mol} \mathrm{~m}^{-2} \mathrm{~s}^{-1}$ and a photoperiod of $16 / 8 \mathrm{~h}$ of light/dark. Full randomisation of light treatments was operated before each experiment. Each experiment was closed when commercial harvest was reached, at 18 Days After light Treatment (DAT), which meant 39 Days After Sowing (DAS).

At harvest time, for all experiments, edible fresh weight (FW) and water use for each plant were measured. Water Use Efficiency (WUE), as the ratio between FW and the volume of water used (g FW L- ${ }^{-1} \mathrm{H}_{2} \mathrm{O}$ ) was calculated. Lighting Energy Use Efficiency (EUE) was determined according to the crop cycle length and the final FW, related to the lamps' cumulated electricity absorption ( $\mathrm{g} \mathrm{FW} \mathrm{kW}^{-1}$ ). Land Surface Use Efficiency (SUE) was determined by analysing the potential achievable yield per unit land surface ( $1 \mathrm{~m}^{2}$, with plant density of 100 plants $\mathrm{m}^{-2}$ ) over a year. Crop Nutrient Use Efficiency (NUE) was calculated by the ratio between FW and the total concentration of selected nutrients ( $\mathrm{N}, \mathrm{P}, \mathrm{K}, \mathrm{Ca}, \mathrm{Mg}, \mathrm{Fe}$ ), analysed in leaf tissues.

Measurements of stomatal conductance ( $\mathrm{mmol} \mathrm{m}{ }^{-2} \mathrm{~s}^{-1}$ ) were carried out on the third fully expanded leaf from the apex using a leaf porometer (AP4, Delta-T Devices). Leaf chlorophyll content was estimated using a hand-held leaf chlorophyll meter ( N -Tester, Yara) on the third fully expanded leaf from the apex. For the determination of total phenolic, flavonoid content and total antioxidant capacity, an extraction of the phenolic compound was performed twice on frozen samples of basil leaves. Total phenolic content (TPC) was determined according to the FolinCiocalteu colorimetric method [6], while total flavonoids content (TFC) was determined by aluminium chloride colorimetric assay [6]. Total antioxidant capacity was measured by Ferric Reducing Antioxidant Power (FRAP) assay [6]. Micro- and macro-nutrient concentrations were measured in basil leaves collected at harvesting. Micro- and macronutrients analyses were performed by using an inductively coupled plasma optical emission spectrometer ICP-OES equipped with a plasma source and an optical detector with a charge-coupled device CCD (SPECTRO Analytical Instruments $\mathrm{GmbH} \& \mathrm{Co}$.) [6]. Total nitrogen (TN) analysis were performed in dry leaf samples by using a thermo-electron CHNS-O elemental analyser.

Data were analysed by two-way ANOVA (light spectrum x experiment) and the means were compared by Least Significance Difference (LSD), at 5\% significance level.

## 3. RESULTS AND DISCUSSION

Considering that no significant interactions between light and experiments were found, average values from the four experiments were used.

Basil FW was enhanced by the increasing RB ratio, reaching the highest values at $\mathrm{RB} \geq 2$ (Table 1). Yield reduction under prevalent blue light was also previously reported [7], due to lower internode growth and smaller plant canopy. A consistent trend in plant chlorophyll content was also evident, with the highest values found when plants were grown at $\mathrm{RB} \geq 3$ (Table 1). Water use per plant varied from $0.34\left(\mathrm{RB}_{1}\right.$ and $\left.\mathrm{CK}_{1}\right)$ to $0.42\left(\mathrm{RB}_{0.5}\right.$ and $\left.\mathrm{RB}_{4}\right)$ and $0.48\left(\mathrm{RB}_{2}\right.$ and $\left.\mathrm{RB}_{3}\right) \mathrm{L}$ plant ${ }^{-1}$ (data not shown). Considering the plant capacity to transform resources, WUE was the greatest in plants grown under RB ratio of 2 or 3 (average value of $44.5 \pm 1.2 \mathrm{~g} \mathrm{FW} \mathrm{L}^{-1} \mathrm{H}_{2} \mathrm{O}$ ), thanks to a higher biomass production despite a slight increase in water use. A greater presence of blue light in the spectrum resulted in higher stomatal conductance (Table 1), as previously reported in other researches [8], due to the action on phototropins, which regulate stomata opening.

Light energy used was highest in $\mathrm{CK}_{1}$ as compared with LED treatments, and, among LEDs treatments, an increase in electricity consumption was associated with the increase in the red portion of the spectrum (data not shown). As a consequence of the increased biomass, EUE was highest with $\mathrm{RB} \geq 2$ (average value of $32.7 \pm 1.0 \mathrm{~g} \mathrm{FW} \mathrm{kW}^{-1}$ ).

Land surface use efficiency was highest when plants were grown with $\mathrm{RB} \geq 2$ (Table 1). When using a vertical system of 5 or 10 layers, daily yield per surface of land occupied would increase up to, respectively, 567 and $1135 \mathrm{~g} \mathrm{~m}^{-2}$ day $^{-1}$, if $\mathrm{RB} \geq 2$ were used (data not shown). These results are very interesting if compared with previous average growth rate of about $24-110 \mathrm{~g} \mathrm{~m}^{-2} \mathrm{day}^{-1}$ recorded in traditional greenhouse production [9-10].

Based on the overall mineral content per plant, N, P, K, Ca, Mg, and Fe were the highest in $\mathrm{RB}_{3}$. From a resource use perspective, NUE was the lowest when $\mathrm{CK}_{1}$ or LED lights with $\mathrm{RB} \geq 2$ were used, and this was associated with higher concentration in leaf tissue of the elements.

The highest FRAP values were found in plant grown under $\mathrm{RB}_{2}$, although with comparable values to $\mathrm{RB} \geq 3$ (Table 1). Total flavonoid content was highest in $\mathrm{RB}_{3}\left(1.60 \mathrm{mg} \mathrm{CE} \mathrm{g}{ }^{-1} \mathrm{FW}\right.$ ) (Table 1). No statistical differences in polyphenols content were observed in response to R:B ratio (data not shown). If the increased antioxidant capacity and flavonoids concentrations are combined with the observed yield increase associated with $\mathrm{RB}_{2}$ and $\mathrm{RB}_{3}$, the number of functional compounds achievable per plant is far greater compared with the other treatments. These results are similar to those found in previous researches [11], where red light was also shown to improve antioxidant capacity in basil.

## 4. CONCLUSION

A R:B ratio of 3 allowed to achieve highest yield, quality and resources use efficiency. At $\mathrm{RB}_{3}$ a reduction in stomatal conductance combined with preserved plant growth resulted in increased water use efficiency. Concurrently, the higher yield associated with $\mathrm{RB}_{3}$ resulted in greater energy use efficiency. Starting from the promising results associated with $\mathrm{RB}_{3}$, future researches should target the effect of additional spectral regions, the identification of crop intra- and inter-specific variability in the response, or the definition of optimal light intensities.

## 5. REFERENCES

[1] Kalantari, F., Mohd Tahir, O., Mahmoudi Lahijani, A., Kalantari, S. 2017. A review of vertical farming technology: a guide for implementation of building integrated agriculture in cities. Adv. Eng. Forum 24: 76-91.
[2] Kozai, T., Niu, G. 2016. Plant factory as a resource-efficient closed plant production system. In: Plant Factory: An Indoor Vertical Farming System for Efficient Quality Food Production, $1^{\text {st }}$ ed. T. Kozai, G. Niu, and M. Takagaki, eds. (Academic Press, Cambridge, MA, U.S.A.). Pp.69-90.
[3] Dou, H., Niu, G., Gu, M., Masabni, J. G. 2018. Responses of sweet basil to different daily light integrals in photosynthesis, morphology, yield, and nutritional quality. HortScience 53: 496-503.
[4] Graamans, L., Baeza, E., Van Den Dobbelsteen, A., Tsafaras, I., Stanghellini, C. 2018. Plant factories versus greenhouses: comparison of resource use efficiency. Agric. Syst. 160: 31-43.
[5] Lin, K. H., Huang, M. Y., Huang, W. D., Hsu, M. H., Yang, Z. W., Yang, C. M. 2013. The effects of red, blue, and white lightemitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (Lactuca sativa L. var. capitata). Sci. Hortic. 150: 86-91.
[6] Pennisi, G., Blasioli, S., Cellini, A., Maia, L., Crepaldi, A., Braschi, I., Spinelli, F., Nicola, S., Fernandez, J.A., Stanghellini, C., Marcelis, L.F.M., Orsini, F., Gianquinto G. 2019. Unraveling the Role of Red:Blue LED Lights on Resource Use Efficiency and Nutritional Properties of Indoor Grown Sweet Basil. Front. Plant Sci. 10: 305.
[7] Chen, X. L., Guo, W. Z., Xue, X. Z., Wang, L. C., Qiao, X. J. 2014. Growth and quality responses of 'Green Oak Leaf' lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). Sci. Hortic. 172: 168-175.
[8] Hogewoning, S. W., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W., Harbinson, J. 2010. Blue light doseresponses of leaf photosynthesis, morphology, and chemical composition of Cucumis sativus grown under different combinations of red and blue light. J. Exp. Bot. 61: 3107-3117.
[9] Saha, S., Monroe, A., Day, M. R. 2016. Growth, yield, plant quality and nutrition of basil (Ocimum basilicum L.) under soilless agricultural systems. Ann. Agric. Sci. 61: 181-186.
[10] Montesano, F. F., van Iersel, M. W., Boari, F., Cantore, V., D'Amato, G., Parente, A. 2018. Sensor-based irrigation management of soilless basil using a new smart irrigation system: effects of set-point on plant physiological responses and crop performance. Agric. Water Manag. 203: 20-29.
 or wavelength-dependent photoresponse of antioxidants in herb microgreens. PLoS One 11: e0163405.

Table 1. Fresh weight, water use efficiency (WUE), Energy Use Efficiency (EUE), land Surface Use Efficiency (SUE), nutrient use efficiency (NUE) chlorophyll content, stomatal conductance, FRAP and flavonoids concentrations of basil plants grown under LED lights with different R:B ratio in the spectrum $\left(\mathrm{RB}_{0.5}, \mathrm{RB}_{1}, \mathrm{RB}_{2}, \mathrm{RB}_{3}\right.$, and $\mathrm{RB}_{4}$ ) or under fluorescent lamps ( $\mathrm{CK}_{1}$ ). Different letters indicate significant differences at $\mathrm{P} \leq 0.05$.

|  |  | RB0.5 |  | RB1 |  | RB2 |  | RB3 |  | RB4 |  | CK1 |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Fresh weight | g plant ${ }^{-1}$ | 12.4 | c | 13.3 | b | 20.5 | a | 21.5 | a | 19.3 | a | 14.7 | bc |
| WUE | $\begin{aligned} & \mathrm{g} \text { FW } \quad L^{-1} \\ & \mathrm{H}_{2} \mathrm{O} \end{aligned}$ | 32.6 | c | 38.5 | b | 43.6 | ab | 45.4 | a | 40.9 | b | 40.6 | b |
| EUE | ${ }_{1}^{\mathrm{g}} \mathrm{FW} \mathrm{kW}$ - | 27.9 | b | 26.9 | b | 33.7 | a | 33.2 | a | 31.1 | a | 13.2 | c |
| SUE | $\underset{\mathrm{d}^{-1}}{\mathrm{~g} ~ \mathrm{FW} ~ m} \mathrm{~m}^{-2}$ | 68.9 | c | 74.1 | b | 113.7 | a | 119.4 | a | 107.5 | a | 81.4 | bc |
| NUE | g FW $\mathrm{g}^{-1}$ nutrients | 186.1 | a | 179.0 | a | 165.5 | b | 154.9 | b | 163.3 | b | 165.2 | b |
| Chorophyll content | N-tester value | 408.9 | c | 437.3 | c | 496.4 | b | 546.0 | a | 527.9 | ab | 431.5 | c |
| Stomatal conductance | $\mathrm{mmol} \mathrm{~m}_{\mathrm{s}^{-1}}^{-2}$ | 210.1 | a | 179.2 | ab | 147.5 | b | 139.6 | b | 159.7 | b | 160.1 | b |
| FRAP | $\begin{aligned} & \mathrm{mmol} \\ & \mathrm{Fe}^{2+} \\ & \mathrm{FW} \end{aligned}$ | 0.7 | b | 0.7 | b | 1.1 | a | 1.0 | ab | 0.8 | ab | 0.7 | b |
| Flavonoids concentration | $\begin{aligned} & \mathrm{mg} \mathrm{CE} \mathrm{~g}^{-1} \\ & \mathrm{FW} \end{aligned}$ | 1.0 | c | 1.2 | bc | 1.3 | b | 1.6 | a | 0.9 | c | 0.9 | c |

