Article

The Growth and Development of Sweet Basil (Ocimum basilicum) and Bush Basil (Ocimum minimum) Grown under Three Light Regimes in a Controlled Environment

Mohammed Aldarkazali, Hail Z. Rihan *, Demelza Carne and Michael P. Fuller

School of Biological and Marine Sciences, University of Plymouth, Plymouth PL4 8AA, UK; mohammed.aldarkazali@plymouth.ac.uk (M.A.); demelza.carne@plymouth.ac.uk (D.C.); mfuller@plymouth.ac.uk (M.P.F.)

* Correspondence: hail.rihan@plymouth.ac.uk; Tel.: +44-7513724273

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Abstract: Light is a crucial element for plant growth and production. In areas where natural light is not sufficient for optimal plant growth and production, high pressure sodium (HPS) light sources are widely used. However, HPS lamps are considered not very electrically efficient generating high radiant heat and as a consequence, there has been a lot of interest in replacing HPS lamps with new more efficient lighting sources in the form of light-emitting diodes (LEDs). The effects of three lighting sources (White LED, Blue/Red LED and HPS) on the growth, development and on the essential oil yield and quality of sweet basil and bush basil were investigated. There was a clear advantage to the Blue/Red (452 nm and 632 nm, respectively) LED on virtually all growth and physiological parameters measured for both basil species. The HPS lighting system always performed least effectively in all comparisons. Combining increases in plant yield and increases in oil yield the Blue/Red LED array outperformed the HPS lights by a factor of approximately double, with the white LED being intermediate between these two extremes.

Keywords: LEDs (light-emitting diodes); high-pressure sodium lamps (HPS); basil; essential oil; photosynthesis; light spectrum

1. Introduction

Ocimum basilicum L. (Basil) belongs to the Lamiaceae family and grows wild in tropical and subtropical climates [1]. Basil is an important culinary herb and essential oil crop grown and used worldwide [2,3] and basil essential oil has been used widely in the food industry as a food flavor, in the medical industries [4], as a component of oral health and dental products and also in the fragrance industry [5]. Moreover, basil has been claimed to be effective in treating several medical complaints such as anxiety, stomach aches, pyrexia, kidney failure, arthropod stings, sickness, infections, headaches, coughs, and constipation [5–9]. Basil 1 is used as flatus-relieving medicine for gastrointestinal disorders and found to increase milk production in nursing mothers [10].

Basil essential oils contain a wide array of chemical compounds, depending on genotype and growing conditions (light, temperature, and irrigation) [11]. The main active biochemical components of basil essential oils are; estragole, a phenylpropane used in perfume manufacturing and as a food additive for flavor [12,13] and linalool, which is used widely as a scent in many hygiene products and cleaning agents [14].
Plants do not utilize all wavebands of white light (sunlight) equally but those between 400–700 nm provide photons of the correct energy to drive photosynthesis and these wavelengths are typically referred to as Photosynthetically Active Radiation (PAR) [15–17]. Other wavebands are also important in photo-morphological development (above 700 nm) and in causing damage to plant cell DNA (below 400 nm) [18].

In high latitudes, especially in the northern hemisphere, warm climate crops such as tomatoes, cucumbers and peppers are grown under protected cropping in glasshouses or poly-greenhouses and supplementary lighting is necessary in order to maintain sustainable rates of growth and production, especially in the winter when natural daylight is limited [19]. High-pressure sodium (HPS) lamps are currently the most commonly used source of supplementary lighting. High-pressure sodium (HPS) lamps emit light over a broad, unmodifiable spectrum of wavelengths but with high peaks in the PAR region. They emit a high degree infrared radiation leading to thermal emission and raising leaf and air temperature and this also reduces the efficiency of these lamps in terms of electrical cost for PAR generation. The heat production from HPS lamps has been found to have a negative effect on plant development [20].

Recently, LED arrays have surpassed HPS lights in terms of the electrical cost per photon [21,22] and now LED arrays have a potential for use in agricultural/horticultural production [23]. Light-emitting diodes (LEDs) have a linear photon output with the electrical input current. LEDs are gaining in substantial importance in plant growth industries not only because LEDs can be constructed in arrays large enough to provide sufficient PAR while remaining small in size (few centimeters in diameter) but also emit less heat than HPS lighting lamps [20,24–26]. LEDs are also known for their durability and long operating lifetime (Dickinson, 2007). Wavelength specificity of LED’s is accomplished via changes in chemical makeup of the semiconducting material used [27]. An increasing body of research is reporting the role of LEDs in enhancing the shape, edible quality [28], biomass, number of leaves [29], growth and stem width and other traits of various plant species [30].

Since individual LEDs only emit low levels of light, arrays of several to hundreds are constructed to boost the overall light output of lighting devices. Furthermore, each individual LED has a narrow waveband, the construction of arrays provides the manufacturers of lighting devices with an almost infinite combination of different LEDs to produce a unit with a very specific overall wavelength pattern. Many researchers across the world are experimenting with different unit arrays in order to determine which LED combinations are most useful as a supplementary or as a sole source light for plant growth.

The current research aimed to compare three commercially available lighting units: HPS, full spectrum LED and Blue/Red LED used as sole source lighting, on the growth and physiological parameters including: plant height, leaf area, fresh and dry weight, stomatal conductance, maximum photosynthesis rate, and chlorophyll fluorescence ratio (as a measure of photosynthetic efficiency) of two species of basil: Sweet basil and bush basil. Moreover, it aimed to investigate the effect of these lighting units of the quantity and quality of essential oil from sweet basil.

2. Materials and Methods

Sweet basil (Ocimum basilicum) and bush basil (Ocimum minimum) seeds were obtained from Suttons seeds Ltd. (UK). Four replicate pots per species were used in each light treatment and with two experimental replications. The treatments were set up in a controlled environment (CE) room divided into three compartments and the treatments randomly allocated to the compartments. In the experimental repeat, the treatments were re-randomized to the compartments. The pots (9 × 9 cm) used in the experiment were filled with 250 g of compost (John Innes No.1) and four seeds were sown in each pot. After sowing the seeds, each pot was watered with 1000 mL of water. The pots were randomly distributed within each compartment. Temperature and humidity were monitored using Gemini data loggers (Tinytag Plus, No GP-1590-, Chichester, UK). The air temperature in the CE room had a set-point of 28 °C. The dark/light period was set to 8 H dark/16 H light. The three-light units used were: High-Pressure Sodium lamp (HPS) (Omega, Barry, UK), White (full spectrum) LED (W LED) (Skyline 400, LED Hydroponics, Berkshire, UK) and Blue/Red (BR) lighting LED (Mars
Hydro, Mars II 1600, Shenzhen, China). Light intensity for all of the lighting units (HPS, W LED, and BR LED) was monitored and adjusted using a Skye PAR quantum sensor (Skye Instruments, Powys, UK) to provide 470 ± 20 µmol m⁻² s⁻¹ at the upper surface of the plant pots. The light spectra were recorded using an UPRtek MK350N premium stand-alone handheld spectral light spectrometer (Figure 1). The HPS lamp showed a broad spectrum of wavelengths with the main radiation peak at 596 nm yellow, W LED showed three main wavelength peaks; 451 nm Blue, 550 nm Green, and 620 nm Red with a significant amount of light distributed in the Green region whilst the BR LED had two main peaks at 459 nm Blue and 632 nm Red.

Growth and physiological parameters were measured at two stages of development; at the maximum leaf development stage (40 days after sowing) and at full flowering stage (54 days after sowing) (harvest 1 and harvest 2). The plants were continuously monitored and irrigated when needed. Extra air circulation had to be provided to plants grown under HPS light unit to maintain the same temperature as other treatments because of heat gain from the lamp and this meant that more frequent irrigation was needed in these pots. Plants were grown for a total of 48 days.

Physiological measurements included; light-saturated instantaneous maximum photosynthetic rate Amax (µg cm⁻² s⁻¹) measured using an LCi-SD Highly Portable Ambient Photosynthesis System (ADC BioScientific, Herts, UK); the chlorophyll fluorescence ratio (Fv/Fm) measured using a Hansatech Pocket PEA meter (Hansatech Ltd, Norfolk, UK); stomatal conductance (Gs mmol m⁻² s⁻¹) measured using a Delta-T AP4 Leaf Porometer (Delta T Devices, Cambridge, UK).

Morphological measurements included; plant height (cm) from the compost surface to the top of the plants; leaf area LA (mm²), using leaf an area image analyser HITACHI KP-D40 color digital camera with a lightbox and WinDias 1.5 software (Delta-T Devices Ltd, Cambridge, UK); fresh weight (FW) and dry weight (DW) (g) after removing the root system, using a sensitive Fisher Scientific SG-402 laboratory balance; plants were dried at 60 °C for 96 h [31].

**Figure 1.** Spectra of the lighting units used as measured by UPRtek. (A) The radiant density of the light spectrum intensity. (B) The relative light intensity.
2.1. Chemical Analysis

Dry plant material (stems and leaves) was used to extract the essential oil from sweet basil using a solvent extraction method [32] with some modifications. Dry plant material (20 g) was ground in a mortar and pestle with 10 mL of FISHER Scientific’s HPLC grade C6H14 (hexane) ≥ 95% (Thermos Fisher Scientific, Gloucester, UK). The resultant mixture was then added to a sintered column with the addition of 60 mL of Hexane. The mixture was then drained and the solution collected. A significant amount of the hexane was evaporated using a BÜCHI R-124 Rotary Evaporator System. A blow-down technique using thermal Techne® Sample Concentrator and BOC Nitrogen gas was applied to evaporate the remainder of the hexane from the solution [33]. The essential oils of sweet basil were then collected in a vial. The vial was weighed after the blow-down to calculate the quantity of the essential oil obtained.

2.2. Gas Chromatography (GC)

Five main components of basil essential oil, according to their common appearance in the literature [34–36] and their industrial importance [37], were isolated and measured: estragole C10H12O, eugenol C10H12O2, (Ethyl) E-cinnamate C11H12O2, eucalyptol C10H18O, and linalool C10H18O.

Reference samples of the five compounds were obtained from SIGMA-ALDRICH with a purity of ±99% and a diluted solution was prepared to a concentration of 250 mg mL⁻¹ with hexane to be compatible with the gas chromatograph (GC) and were used as standards for the GC analysis. GC analysis was conducted according to the procedure of Chenni et al. and Kumari et al. [38,39]. The GC was performed using a 7890B Gas Chromatograph System. The essential oil of sweet basil (0.025 g) was added to 10 mL hexane to make a stock solution. A working solution was then prepared by adding 1 mL of the stock solution to 9 mL of hexane. A solution of 1–1.5 mL was added to GC vial.

Stock solutions of the reference samples were prepared using the same procedure: 0.1 mL of each stock solution of each chemical (five chemicals in total) added together in a single GC vial to give a total of 0.5 mL. Then 0.5 mL of hexane was then added to the GC vial to make a final test solution of 1 mL solution of 250 mg mL⁻¹ concentration.

A solution of 1–1.5 mL hexane was added to each GC vial and then analyzed using an Agilent Gas Chromatograph 7890A equipped with a 7683 Series Autosampler and 7683B Series Auto-injector (Agilent, Santa Clara, CA 95051, USA) and HP5 Column, 5% phenyl-95% dimethylsiloxane, low polarity column, 30 m × 0.320 mm i.d., and 0.25 μm film thickness. The carrier gas was nitrogen at a flow rate = 1.0 mL min⁻¹, Injector temperature was 250 °C, Flame Ionization Detector temperature was 300 °C, Hydrogen flow rate was 40 mL min⁻¹, Airflow rate = 400 mL min⁻¹, Nitrogen (make-up) flow rate = 15 mL min⁻¹. General Method temperature was programmed at 40–300 °C at 10 °C min⁻¹, 10 min at 300 °C using ChemStation software (Revision B.03.01, May 2007).

2.3. Statistical Analysis

Results are presented as means ± standard error (S.E.). All data were subjected to analysis of variance (ANOVA) using Minitab software (version 17) and comparisons of means were made using the least significant difference test (LSD) at 5% level of probability.

3. Results

3.1. Physiological Responses

3.1.1. Maximum Photosynthetic Rate $A_{\text{max}}$

There was a significant effect of the lighting treatments on the light-saturated instantaneous maximum photosynthetic rate $A_{\text{max}}$ of sweet basil ($p = 0.005$) and bush basil ($p = 0.001$). The highest $A_{\text{max}}$ was observed in plants grown using BR LED lights at both harvest stages. There was no significant impact of the harvest stage and there was no interaction between the lighting treatments and harvest stages on the $A_{\text{max}}$ (Figure 2).
3.1.2. Stomatal Conductance $G_s$

There was a significant effect of light treatments and harvest stage on the stomatal conductance $G_s$ in sweet and bush basil ($p \leq 0.001$). Moreover, there was a significant interaction between the light treatments and harvest stage on $G_s$ for both plant species. The highest $G_s$ was observed under the BR LED lighting unit at both harvest stages and in both plant species (Figure 3).

3.1.3. Chlorophyll Fluorescence Ratio $F_v/F_m$

There was a significant effect of the light treatments on the chlorophyll fluorescence rate $F_v/F_m$ for sweet basil ($p = 0.001$) but not for bush basil ($p = 0.1$). The highest $F_v/F_m$ was observed in plants grown under the BR LED light treatment in sweet basil for both harvest stages (Figure 4). There was a significant effect of the harvest stage on $F_v/F_m$ in bush basil ($p = 0.02$) and $F_v/F_m$ at the first harvest was significantly higher than at the second harvest. However, for sweet basil, the harvest stage had no significant effect on $F_v/F_m$ ($p = 0.2$).
3.2. Morphological Responses

3.2.1. Height

There was no significant effect of the light treatments on plant height in both sweet and bush basil. The harvest stage has a significant effect on the plant height ($p \leq 0.001$) with taller plants observed at harvest stage two (Figure 5).

3.2.2. Leaf Area LA

In sweet basil, there was a significant effect of the light treatments and harvest stage on the leaf area LA ($p \leq 0.001$). The optimum LA was observed in plants grown under the BR LEDs at both harvest stages for both species. For bush basil, there was a significant effect of the light treatments ($p = 0.02$) on LA and plants cultivated under BR LEDs showed larger leaf area per ground surface area unit. LA was significantly higher ($p \leq 0.001$) at harvest stage two in both plant species (Figure 6).
3.2.3. Fresh Weight (FW)

There was a significant impact of light treatments on the fresh weight FW of sweet basil ($p = 0.006$). The maximum FW was observed in plants grown under BR LED lighting unit at both harvest stages for both plant species. There was a significant effect of the harvest stage ($p \leq 0.001$) on FW. At harvest stage two FW was significantly higher in comparison with harvest stage one for both Sweet and bush basil.

In terms of bush basil, there was a significant interaction between light treatments and harvest stage ($p = 0.01$). While the highest fresh weight was observed in plants grown under White LEDs at harvest stage one, FW was highest in plants grown under BR LEDs at harvest stage two (Figure 7).

3.2.4. Dry Weight DW

There was no significant impact of the light spectrum on DW of sweet basil but there was a significant effect on bush basil ($p = 0.001$) under BR LEDs (Figure 8).

The harvest state had a significant impact on DW for both plant species and was significantly higher at harvest stage two.
3.2.5. Essential oil Yield and Composition

Light treatments had a significant impact on the yield of essential oil in sweet basil \((p = 0.005)\). The highest essential oil yield was observed in plants cultivated under BR LEDs (Figure 9).

Results from the Gas Chromatography analysis showed that LED lighting significantly improved the accumulation of linalool, Eugenol and E-Cinnamate in the essential oil of sweet basil compared to the accumulation of those biochemicals in the essential oil of plants grown under HPS lamp. Moreover, BR LEDs significantly increased the concentration of Eucalyptol in contrast with both White LED and HPS lights (Figure 10).
4. Discussion

This study clearly demonstrated that different lighting systems with the same PAR output but with different spectral profiles can influence the growth and yield of basil plants and the yield of essential oil. This correlates well with several other studies [40–43]. A consistent and strong trend can be observed through all of the results in favor of LED arrays in comparison to HPS lights and in favor of the BR LED in comparison to the White LED array. The physiological measurements made indicated that photosynthesis was more productive under the LED arrays with higher Amax and Fv/Fm and this appears to have resulted in greater leaf area production leading to greater growth and yield. Gaining this sort of knowledge of plants growth and morphology in response to lighting changes gives a better understanding of more efficient cultivation of these plants. In particular, controlled environment cultivation of plants increases the chances of enhancement and uniformity in yield and quality but standard controlled conditions for each plant species needs to be carefully optimized. [44]. Precise regulation of environmental control in commercial controlled environments can be considered to still be in its early stages since each plant species may have a different response to different light quality and spectra, photoperiods and light integrals as it passes through its developmental stages.

These considerations must be observed when switching the cultivation of plants from field to any indoor farming unit and monitoring the photosynthetic activity is an important tool to achieve that goal. Mimicking the sunlight energy efficiency is not an easy task but has been established that the effect of Red light in the region of 650–665 nm from LED lighting units matches the assimilation peak of the photoreceptors phytochrome and chlorophyll. It was shown also, that the combination of Red–Blue light in LED lighting for growing plants, can enhance the Maximum Photosynthetic rate Amax as a consequence of activation of Cryptochromes, Phytochromes and Chlorophyll more than if a monochromatic light was used as a grow light. [45]. This increase in Amax was explained as a result of the increase in carbon dioxide levels when the Blue light caused a better stomatal gapping [46–48]. The addition of Blue LED light in the region (380–495 nm) also stimulates Chlorophyll and is also found to trigger Cryptochromes influences stem growth. This correlates with the results presented here where it was found that Gs was higher under BR LED. This increase in Amax and Gs thus leads to an increase in DW with the addition of Blue light. The value of Amax can respond to the Red light (peak 656 nm) more than a response to a White LED light [49]. On the other hand, it was found that there is a negative effects of the addition of Green LED light (495–570 nm) on the growth and development of plants which supports our findings as White LED and HPS lighting (contain some Green spectrum) promoted lower values of growth than those plants under BR LED light (no green light). Curiously, PAR is defined to include all wavelengths between 400 and 700 nm which of course
includes green light which is not very photosynthetically active. This challenges the set-up of experiments designed to compare lighting units with each other. If for example, as used here, the PAR is set to be similar under all light units, by the above definition, those containing green light will be proportionately lower in the required red and blue light. Such considerations may require a re-examination of the instruments used to measure light in LED lit controlled environments with a possible move away from Quantum Sensors towards Spectrometers which can measure specific wavelength intensities more accurately.

In the process of photosynthesis, Photosystem II (PS II) light-dependent chemical reaction productivity rate (Fv/Fm) can be measured as an indication of a healthy plant [50,51]. Fv/Fm is a normalized ratio created by dividing variable fluorescence by maximum fluorescence. It is a measurement ratio that represents the maximum potential quantum efficiency of Photosystem II when all reaction centers are open [51]. Fv/Fm is sensitive to the light conditions and therefore lower values of Fv/Fm can indicate stress via photo-inhibition. Fv/Fm is related to the quantum yield in PS II as other studies have shown similar results where Blue LEDs increased photochemical quenching whilst Red LEDs enhanced chlorophyll content despite chloroplasts being smaller [52]. The current analysis has shown that a combination of Blue (459 nm) and Red (632 nm) LED lights can promote a higher Fv/Fm than HPS or white LED lights of the same PAR output. Moreover, Fv/Fm was around 0.8 (optimal) even at harvest stage two using the BR LEDs (Figure 2) whereas it had diminished to less than optimal under the other light treatments. This is an indication that the BR LEDs provides suitable conditions for efficient photosynthetic activities to persist at late growth stage of the plant [45,53,54].

The effects of light quality on photosynthesis go parallel with the effect on stomatal gapping. It has been established that Blue region of the spectrum has more impact on the stomatal opening than Red light [55,56]. Both of these studies showed the low impact of green light on stomatal opening. Red and blue wavelengths are both utilized during photosynthesis by chlorophyll and secondary pigments, therefore promoting growth and development in plants [52,54]. A recent study sought to un-pick the influences of Red and Blue light on growth and photosynthetic efficiency in sweet basil and found that quantum efficiency in PSII was lower under Red/Blue LED light arrays when the Red portion of the light array was greater than double the Blue (2:1). This was reversed when light ratios were more equal (1:1) [57]. It is the presence of the Photosystem II Primary Donor protein P680 in PSII which absorbs maximum light energy at 680 nm (Red) and Photosystem I Primary Donor P700 in PSI which absorbs maximum light energy at 700 nm (Red) and these might be responsible for the effects on the stomatal conductance Gs [58–60]. In the current study, the ratio of Blue:Red in BR light treatments was higher than the ratio of Blue; Red in both White LED and HPS lighting units. This could explain the high Gs value results under BR LED as a response to the high Blue; Red ratio.

An increase of Blue light had a positive effect on lettuce a negative impact on soybean and wheat [61]. In contrast, sweet basil was positively affected by Blue light when measuring FW (at 200 μmol m⁻² s⁻¹) and when the same light quality compared with low light intensity (at 100 μmol m⁻² s⁻¹) the FW value was lower indicating the importance of combinations of wavelengths and quantity to promote high yielding plants [62]. Light has a relationship with FW of plant material and it has been found that Blue light (455–475 nm) with light intensity (140 ± 10 μmol m⁻² s⁻¹) resulted in high FW [63].

The highest LA value was observed under Blue/Red lighting treatments in comparison with both white LED and HPS lighting units. In agreement with current findings, the light spectrum of 580 nm, 600 nm was reported to inhibit the growth and development of leaves in Lettuce leading to a low value of LA [64]. However, in disagreement with the current research, it was reported that an increase of Blue light 455 nm by 6.7 μmol m⁻² s⁻¹ over 400 μmol m⁻² s⁻¹ for 20 h decreased the LA of tomato (Solanum lycopersicum) and Cucumber (Cucumis sativus) [65]. The difference in plant heights and LA response to light quality could be due to the effect of plant species and experimental conditions.

Alongside the main photo-related photosynthesis reaction, a phenyl-based anabolism reaction accrues in plant species resulting in organic matter that gives plants their specific aroma and taste. Many research reports have indicated the impact of light quality on the essential oils of plant species.
and the current data agrees that light composition has an effect on the quantity and quality of essential oil of sweet basil. BR LED regime had a significant positive impact on the quantity and quality of essential oil extracted from sweet basil and this might alter the taste these plants. In another study, the essential oil content of basil grown under Blue light has been found to be up to four times higher than those grown without Blue light [66]. This is in agreement with our findings where BR LEDs had a positive impact on the level and concentration of important biochemicals in the essential oil of sweet basil.

5. Conclusions

Light quality had an impact on the physiological and growth characteristics of sweet basil and bush basil. LEDs lighting devices had an advantage in promoting high yielding plants with a higher concentration of essential oil compared to traditional HPS lamps. There was a clear advantage of using a Blue-Red LED lighting unit (452 nm Blue 632 nm Red) compared to a white LED unit; this research could have a great practical application for the commercial production of basil in sole source-controlled environments. Further research is recommended to determine more precisely the optimal wavelength of light in both the Blue and Red regions and the optimal Blue/Red combination for the growth of and production of basil.

Author Contributions: M.A.: conducted the laboratory experiments; H.Z.R.: supervised M.A. thorough the laboratory experiments, helped with results and statistical analysis and writing up the manuscript; D.C. helped in running the laboratory experiments. She also helped in revising the manuscript; M.P.E.: The research took place in Mick Fuller plant physiology laboratory. M.P.F. helped in supervising the whole research. He also helped in writing up the manuscript and proof-reading the English.

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