



RESEARCH ARTICLE

Effect of Different Light Colors (Red, Blue, and Green) on the Growth and Physiological Performance of Seedlings

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ABSTRACT

Photosynthesis, morphogenesis and biochemical composition of plants is controlled by an environmental parameter (light) is the presence of a set of photoreceptors. The objective of the present study was to assess the effect of illumination with red (660 nm), blue (450 nm) and green (530 nm) emitting diode (LED) lights on the growth and physiological performance of common bean (*Phaseolus vulgaris* L.) seedlings as compared to a white-light control under controlled greenhouse conditions. The seedlings were cultivated in the same photon flux density (PFD) ($150 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$) and photoperiod (16 hours of light and 8 hours of dark) for 21 days for each light treatment. Growth parameters were measured such as plant height, leaf number, stem diameter, root length and biomass; physiological indices were measured: total chlorophyll content and carotenoid content. Red light resulted in the highest plant height and internode elongation along with the characteristic of auxin related growth, whereas, blue light gave shorter and more compact plants, with significantly higher number of leaves, stem diameter, chlorophyll content and biomass of roots. The lowest growth and chlorophyll content were observed under the green light for all treatments, which has the least absorption by chlorophyll pigments. Intermediate balanced growth was achieved with white light. The present results corroborate the differential effects of red and blue wavelengths on growth and photosynthetic pigment content of the plant, and the role of blue light in producing compact, stress-tolerant growth.

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1. Introduction

In addition to being the main source of energy for photosynthesis, light also serves as a key information signal that regulates the growth, development and morphology of plants, referred to as photomorphogenesis [1]. The plants detect light with the help of special light receptors, such as phytochromes (which are sensitive to the red and far-red part of the spectrum), cryptochromes and phototropins (which are sensitive to blue and UV-A light) and, to a lesser extent, green light receptors [2]. These photoreceptors control multiple processes such as seed germination, stem elongation, leaf expansion, biosynthesis of chlorophyll, stomatal development and flowering time [3].

There is a growing interest in the effects of different light wavelengths on crop growth and quality with the introduction of light emitting diode (LED) technology in controlled-environment agriculture, vertical farming and greenhouse horticulture [1,3]. LEDs can be used to manipulate light quality, intensity, and photoperiod, which could help to optimize the production of crops for specific desired traits, including compact growth habit, enhanced pigmentation, and increased accumulation of biomass [4]. Red light (600–700 nm) has been found to be more efficiently absorbed by chlorophyll and it has been shown to increase the elongation of stems and biomass

accumulation, but too much red light alone can lead to the 'red light syndrome', which is the term used for the abnormal leaf morphology and decreased photosynthetic efficiency [2,4]. Blue light (400-500 nm) controls stomatal opening, chloroplast development and generally shortens plants, thickens leaves and increases leaf chlorophyll content [5]. Green light (500–600 nm) was historically believed to be non-physiologically active in the sense that it is poorly absorbed by the pigment, but recent research indicates that green light can penetrate deeper into the canopy and have a role in shade-avoidance and gas exchange reactions, though, in general, to a lesser extent than red or blue light [1,3,5].

While there are a number of reports on the use of each single waveband, comparative studies that assess the use of red, blue, and green light under equal- and full-intensity lighting conditions are still useful in making practical decisions in controlled-environment agriculture (CEA). The objectives of this study are to assess the effects of LED lights of different colors (red, blue, and green) on the growth and physiological performance of bean seedlings to that of a white-light control, and to correlate the responses observed with the known photobiological function of each waveband.

Table 1. Experimental light treatments and growth chamber conditions.

Treatment	Light Source (Peak Wavelength)	Photon Flux Density ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Photoperiod
R	Red LED (660 nm)	150 ± 5	16 h light / 8 h dark
B	Blue LED (450 nm)	150 ± 5	16 h light / 8 h dark
G	Green LED (530 nm)	150 ± 5	16 h light / 8 h dark
W (control)	White fluorescent (400–700 nm)	150 ± 5	16 h light / 8 h dark

2.3 Growth Measurements

Leaf number, plant height and stem diameter (measured using a digital caliper at 2 cm above the soil surface) were measured at 3 day intervals for 21 days. The plants were harvested after the end of the experiment and root length was measured. The fresh weight was measured immediately after harvesting and the dry weight after the plant material was oven-dried for 48 hours at 70 °C.

2.4 Chlorophyll and Carotenoid Determination

The Spectrophotometric determinations of total chlorophyll (a + b) and carotenoid content were carried out. The fresh leaf tissue (0.1 g) was extracted in 80% acetone and the absorbance was recorded at 663, 645 and 470 nm in UV-Vis spectrophotometer. The pigment concentrations were determined by standard equations, and reported as mg of pigment per g of fresh weight.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Common bean (*Phaseolus vulgaris* L. cultivar.) seeds. All seeds (including 'Contender') were surface-sterilized in 1% sodium hypochlorite for 5 minutes, then rinsed with plenty of distilled water and germinated in trays filled with a sterile peat moss (3:1) and perlite mix. The seedlings were uniform at emergence (n = 60) and transplanted into separate pots (15 cm) and randomly allocated to one of four light treatments (n = 15 per treatment).

2.2 Light Treatments

The seedlings were raised in individual growth chamber, which were kept separated from each other to avoid cross-contamination of wavelengths. A standard photosynthetic photon flux density (PPFD) of $150 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ was achieved at the canopy height with a quantum sensor, and LED arrays (Red: 660 nm; Blue: 450 nm; Green: 530 nm) and cool-white fluorescent tubes (control) were installed in each chamber. All chambers had a photoperiod of 16 h light / 8 h dark, a temperature of 24 ± 2 °C (day) / 20 ± 2 °C (night), and a relative humidity of 60–70%. The treatment details are summarized below in Table 1.

2.5 Experimental Design and Statistical Analysis

The experiment was laid out in a completely randomized design (CRD) having four treatments and fifteen biological replicates for each treatment. One-way analysis of variance (ANOVA) was used to analyze the data and Tukey's Honestly Significant Difference (HSD) test was used to separate the means at $p \leq 0.05$. The statistical analyses were carried out with the use of the common statistical software. Values are the mean $\pm$ SE, means with different superscript letters within a column differ significantly.

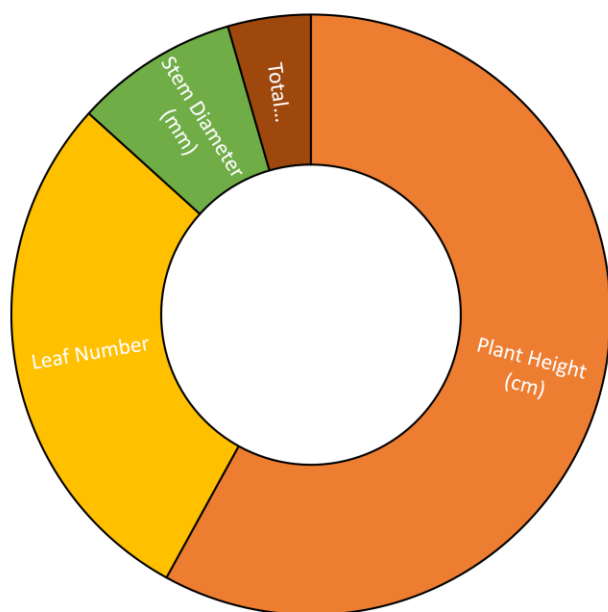
3. Results

3.1 Vegetative Growth Parameters

All the growth parameters studied were significantly affected ($p < 0.05$) by light color. Red light resulted in the tallest plants (18.4 ± 1.2 cm), significantly taller than those in white light (15.9 ± 1.0 cm), blue light (12.6 ± 0.9 cm) and green light (9.8 ± 0.8 cm), which is consistent with the

effect of red light on stem elongation via phytochrome signaling. In comparison, blue light yielded significantly shorter, but more compact plants with maximum leaf number (11.4 ± 0.7) and maximum stem diameter (3.4 ± 0.3 mm) in all treatments. Lower plant height, leaf number and stem diameter were produced under green light indicating less efficiency of green wavelengths in photomorphogenic and photosynthetic response, as compared to red light treatment. In Figure 1 growth and pigment data are summarized.

Figure 1. Growth and pigment parameters of bean seedlings after 21 days under different light-quality treatments



3.2 Chlorophyll and Carotenoid Content

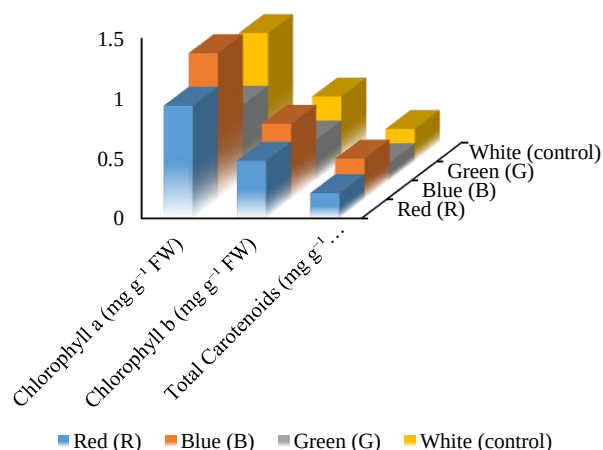
The total chlorophyll content was significantly higher at blue light (1.85 ± 0.11 mg g⁻¹ FW), about 91% higher than the green light (0.97 ± 0.08 mg g⁻¹ FW) showed the lowest amount of chlorophyll among the various treatments. The chlorophyll concentration under white-light and red-light conditions was intermediate (1.61 ± 0.10 and 1.42 ± 0.09 mg g⁻¹ FW, respectively) (Fig. 2). A similar distribution was noted for carotenoid content, with the greatest amount being observed in blue-light-grown plants, an effect that indicates that carotenoid levels may be increased in plants exposed to blue-light-dominated conditions to aid in photoprotection.

3.3 Biomass Accumulation

The seedlings grown under blue light conditions recorded the highest root dry weight and shoot dry biomass, the white-light control had the second highest, while red light

and green light had the third and lowest root and shoot dry biomass, respectively. Root length showed a similar trend, with blue light inducing the longest roots, consistent with the known function of blue light photoreceptors in control of root architecture and carbon allocation belowground.

Figure 2. Chlorophyll and Carotenoid Content after 21 days under different light-quality treatments



4. Discussion

Based on the results of this study it is confirmed and extended that light quality is an important differential factor in the morphological and physiological growth of bean seedlings as reported earlier in the case of horticultural crops. Shade-avoidance response is well documented to be mediated by phytochrome, and the dramatic stem elongation observed under red light is consistent with this, as most of the light in this experiment was absorbed more strongly by chlorophyll than by far-red light, leaving a high red-to-far-red ratio (R:FR) that may still be responsible for elongation responses due to phytochrome photoequilibrium response dynamics and auxin redistribution towards the stem [5,6].

The compact growth habit, increased leaf number and increased chlorophyll and carotenoid levels observed under blue light are consistent with the known activities of cryptochromes and phototropins related to reduced stem elongation and enhanced chloroplast development, stomatal opening and leaf expansion [2,5]. The reason for the better biomass accumulation in this treatment despite the shorter plant growth is not clear, but may be due to the up-regulation of genes related to the biosynthesis of chlorophyll pigments and an increase in the activity of the enzyme Rubisco under blue light. The applied implications of these findings are to support vertical farming systems in which high planting density and increased photosynthetic

performance are desired, which requires the use of light spectra in the blue spectrum [6].

This is not surprising because under green light, chlorophyll a and b have low absorptance i.e. most green light is reflected or transmitted. It is important to note, however, that in natural canopy conditions, green light can reach lower canopy levels than other colors and may have a more significant contribution to the total canopy photosynthesis; this may be underestimated in the present study due to the isolated monochromatic conditions [7]. The intermediate performance of the white-light control (which is a balanced mix of the red, blue and green wavelengths of light) indicates that a combination of these wavelengths of light (combined-spectrum lighting) may moderate extreme morphological responses to monochromatic treatments, and that optimal plant growth under artificial lighting would be best achieved by using combinations of red and blue light (and to a lesser extent, green and far-red light) that have been tailored to the specific growth requirements of the plants [6,7].

4.1 Limitations

This study was done on one species and cultivar in short-term controlled conditions and the responses may vary from one species to another dependent on the shade-tolerance strategy or the sensitivity of the photoreceptors. Moreover, monochromatic light treatments are not able to fully simulate the complex spectrum of light that plants are

exposed to in natural or mixed-LED greenhouse conditions. Combined red-blue-green ratios, dose-response relationships under different photon flux densities and long-term effects on reproductive development and yield should be investigated in future.

5. Conclusion

This study is in agreement with other studies that light quality had a significant effect on plant growth, morphology and photosynthetic pigment content. Red light stimulated stem elongation, blue light stimulated compact plants with high chlorophyll content and high biomass, and green light had the weakest growth response overall. These results corroborate the physiological justification for the red-blue LED formulations commonly used in controlled-environment agriculture and show the special role that blue light plays in the promotion of strong vegetative development. Applications include strategic use of blue enriched spectra for greenhouse and vertical farming to produce compact, quality seedlings, and red light supplementation may be used when a desired plant height or biomass extension is needed.

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Declaration of conflict of interest

The authors no conflict of interest.

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