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Research Article

The effects of LED light intensity on lettuce coloration and biomass

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Light emitting diodes can potentially be a useful tool for lettuce (*Lactuca sativa*) indoor production because of its ability to control light spectra and provide high light levels with little radiant heat. This study was conducted to test coloration, biomass, and anthocyanin concentration in lettuce grown under different light intensities, giving us insights on how changing light intensities can improve coloration and increase productivity. The experiment was conducted in a laboratory with controlled light, humidity and temperature, eliminating as much variability as possible. The following three treatments were used: (1) standard intensity at 250 µmol/m^2/s throughout the growing period, (2) high intensity at 500 µmol/m^2/s throughout the growing period, and (3) standard intensity at 250 µmol/m^2/s then high intensity of 500 µmol/m^2/s two days before harvest. Measurements included subjective visual color ratings, fresh weight biomass, and anthocyanin concentration. There were significant differences among treatments in color ratings and biomass, and no significant differences in anthocyanin concentration. The 250 µmol/m^2/s and 250/500 µmol/m^2/s treatments showed little differences in color rating. The 500 µmol/m^2/s treatment led to higher red pigmentation in plants. The 500 µmol/m^2/s treatment was numerically but not significantly higher in biomass than the 250 µmol/m^2/s treatment, while the 250/500 µmol/m^2/s treatment was lowest. Growers who produce lettuce in a greenhouse setting and are capable of increasing light intensity can apply light intensity at 500 µmol/m^2/s throughout the growing season to improve coloration and maintain productivity.

Keywords: anthocyanin, pigment, Light emitting diodes, color rating, *Lactuca sativa*

Introduction

Anthocyanins in plants are nutritious and beneficial for human health due to their free-radical scavenging and antioxidant capacities (Lila, 2004). Increased anthocyanin content in lettuce can improve both coloration and nutrition of the produce. Light emitting diodes (LEDs) are an emerging light source for crop production under controlled environment with the capacity to control light spectra and provide high light level with little radiant heat (Morrow, 2008). However, the concentration of anthocyanins in lettuce growing under LED lights is low (Park et al., 2012; Pocock, 2016).

Anthocyanin concentrations in lettuce increased at higher light intensities (Kang et al., 2013; Voipio & Autio, 1994). However, biomass accumulation and anthocyanin concentrations are, in general, negatively correlated (Pocock, 2016). To maximize the anthocyanin content in lettuce, both the anthocyanin concentration and biomass need to be enhanced. We hypothesized that using a standard light intensity good for biomass accumulation incorporated with higher light intensity two days before harvest can increase anthocyanin content in lettuce growing under LED lights. The two days of shifting to high light intensity was determined based on a previous research that anthocyanin concentrations reversed after 24 hours shifting the light source (Pocock, 2016). Results of this study can provide guidance for light management to improve lettuce productivity and nutritional quality.

Materials and Methods

Plant materials preparation and management

The study took place in a controlled-environment laboratory in the Agricultural Sciences building at Abraham Baldwin Agricultural College, beginning September 15, 2017. The temperature of the room was set at 23 °C and the relative humidity at 45%. The actual average temperature during the study period recorded by a HOBO® data logger (Onset Computer Corporation, Bourne, MA) was 23±1 °C and relative humidity 58±9%.

One hundred and eight 3 ½" plastic pots were filled with the rooting medium Pro-Mix® BX Mycorrhizae® (Premier Tech, Rivière-du-Loup, Canada). Then nine pots were put in a bottom tray in 3 rows of 3 pots under each light treatment in each block. Each pot was hand seeded with three seeds of 'Outredgeous' lettuce.

All lettuce plants were watered and fertilized equally, regardless of treatments. The first week after planting, each pot was watered with tap water to the rooting medium every day. After the first week and the plants emerged, the plants were watered with tap water to the bottom tray when needed. Miracle-Gro® All Purpose Plant Food 24-8-16 (The Scotts Miracle-Gro Company, Marysville, OH) was mixed (1 teaspoon fertilizer per gallon of water) and applied 14 days after seeding, and once a week afterwards.

Light intensity treatments

The experiment was conducted as a Randomized Complete Block Design with four blocks and three treatments in each block. The three treatments were: (1) standard intensity at 250 µmol/m^2/s throughout the growing period, (2) high intensity at 500 µmol/m^2/s throughout the growing period, (3) standard intensity at 250 µmol/m^2/s and then high intensity at 500 µmol/m^2/s two days before harvest. No ambient light was allowed to plants. The photoperiod was 16 h/8 h (day/night) for all treatments.

Four light stands mounted each with three Triple-Band LED bar lights (Agrivolution LLC, South Windsor, CT) were positioned above the lettuce plants. Light intensity was modified by the distance between the light and plant canopy. Light intensity was checked once a week by a handheld ceptometer (METER Group, Inc., Pullman, WA) and the distance between the light and the plant canopy was adjusted once a week to maintain the constant light intensity for each treatment.

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Data collection

A visually subjective rating of plant color was taken for each pot on 10/20/2017 (35 days after seeding). Color was rated on a scale of 0-100%, representing the redness in leaves. On 10/27/2017 (42 days after seeding), the color rating was conducted by the same person for consistency. Then all lettuce plants were cut using hand sheers to the top of the pot media to harvest the complete aboveground biomass. Lettuce biomass was measured as fresh weight aboveground in grams for each pot.

Then lettuce was combined within each treatment each block for anthocyanin quantification in the Pesticide Formulation, Soil Termiticide, Treated Wood, Use/Misuse, and Groundwater Laboratories at Georgia Department of Agriculture. Anthocyanin concentrations were determined using a modified protocol described by Bumgarner et al. (2012). The pigment was extracted from flash-frozen lettuce tissue samples stored at −20 ◦C. The frozen tissue was homogenized using a processor. Two 5.0 g sub-samples of the homogenized tissue were then extracted twice consecutively with 20 mL and then 20 mL of 1% HCl acidified methanol. Each 1 hour-long extraction took place at room temperature and then samples were centrifuged at 4750 × g for 10 min at approximately 20 ◦C. The supernatant from the two extractions was combined, centrifuged one final time, and then immediately read on a UV-Vis spectrophotometer. Anthocyanin absorbances were obtained at 530 nm. Standard curves for cyanidin-3-glucoside (Chromadex, Irvine, CA, USA) were then used to calculate tissue anthocyanin concentrations from spectrophotometric absorbances.

Data analysis

Data were analyzed by SAS (version 9.4; SAS Institute, Cary, NC) using Proc GLM for analysis of variance (ANOVA) to test for differences in treatment effects, using Tukey's Protected LSD, with significant differences identified at $p<0.05$.

Results

A significant treatment difference was identified using ANOVA for the color ratings on both days and biomass, with p-values less than 0.05 (Table 1). The p-value for the anthocyanin measurement was greater than 0.05 meaning that there were no significant differences among treatments. The average anthocyanin concentration was 0.16, 0.18, 0.15 mg/g fresh weight in the 250 µmol/m^2/s, 500 µmol/m^2/s, 250/500 µmol/m^2/s treatment, respectively.

Table 1. ANOVA results for color ratings on 10/20/2017 and 10/27/2017, biomass on 10/27/2017, and anthocyanin concentrations of lettuce grown under three light intensity treatments.

Measurement	P Value[z]
Color Rating on 10/20/2017[y]	<.0001
Color Rating on 10/27/2017[y]	<.0001
Biomass[x]	<.0001
Anthocyanin Concentration[w]	0.33

[z] There was a significant difference among treatments when p value is smaller than 0.05.
[y] Color was rated on a subjective assessment scale of 0% to 100%, representing the redness in leaves.
[x] Biomass was recorded as lettuce fresh weight aboveground in grams.
[w] Anthocyanin concentration was calculated using a standard curve (r^2=0.999).

Color rating

The 500 µmol/m^2/s treatment had the highest percentage of red pigment ratings on both days (Fig. 1 and 2). The 500 µmol/m^2/s treatment had a 21.96% of red pigment on 10/20/2017 and 26.13% on 10/27/2017, which was about 3x higher on 10/20/2017 and 50% higher on 10/27/2017 than the 250 µmol/m^2/s and 250/500 µmol/m^2/s treatments. The 250 µmol/m^2/s and 250/500 µmol/m^2/s treatments were similar each rating time.

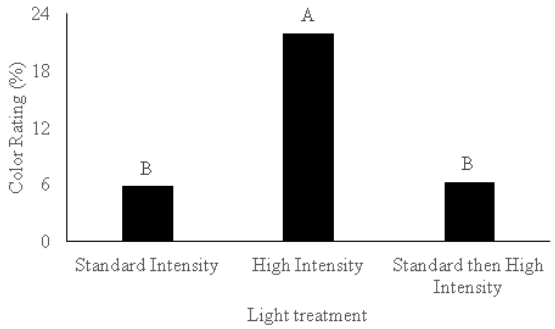

Figure 1. Color rating of lettuce grown under three light intensity treatments on 10/20/17. The standard intensity treatment is equal to 250 µmol/m^2/s of light throughout the growing period. The high intensity is equal to 500 µmol/m^2/s of light throughout the growing period. The standard then high intensity treatment is standard intensity at 250 µmol/m^2/s and then high intensity at 500 µmol/m^2/s two days before harvest. On 10/20/17 each pot was rated on a scale from 0% to 100%, representing the redness in leaves. Bars with the same letter are not significantly different ($p<0.05$) as analyzed using the ANOVA test and then the Tukey's Protected LSD for multiple comparisons in the GLM procedure in SAS.

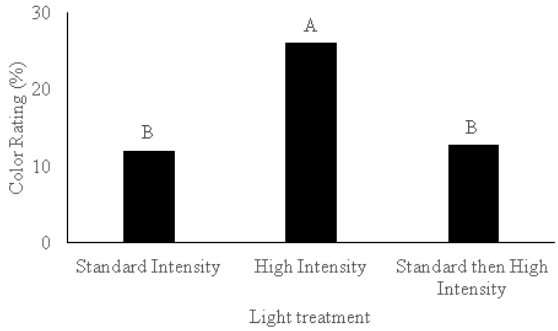

Figure 2. Color rating of lettuce grown under three light intensity treatments on 10/27/17. The standard intensity treatment is equal to 250 µmol/m^2/s of light throughout the growing period. The high intensity is equal to 500 µmol/m^2/s of light throughout the growing period. The standard then

high intensity treatment is standard intensity at 250 µmol/m^2/s and then high intensity at 500 µmol/m^2/s two days before harvest. On 10/27/17 each pot was rated on a scale from 0% to 100%, representing the redness in leaves. Bars with the same letter are not significantly different ($p<0.05$) as analyzed using the ANOVA test and then the Tukey's Protected LSD for multiple comparisons in the GLM procedure in SAS.

Biomass

Overall the change in light intensity caused a lower biomass (Fig. 3). There was no significant difference between the 250 µmol/m^2/s and 500 µmol/m^2/s treatments. The 250/500 µmol/m^2/s treatment had a smaller biomass than the other two treatments.

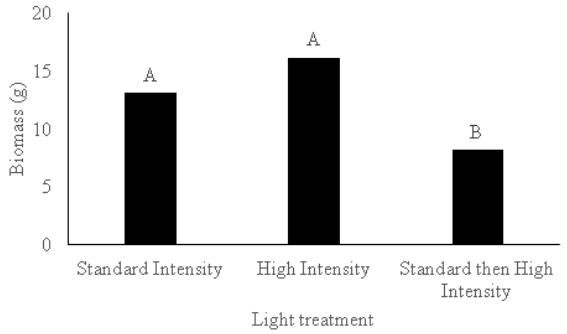

Figure 3. Biomass per pot of lettuce grown under three light intensity treatments. At harvest, lettuce from each pot was cut at the soil level, then put on a scale to record the fresh weight in grams. The standard intensity treatment is equal to 250 µmol/m^2/s of light throughout the growing period. The high intensity is equal to 500 µmol/m^2/s of light throughout the growing period. The standard then high intensity treatment is standard intensity at 250 µmol/m^2/s and then high intensity at 500 µmol/m^2/s two days before harvest. Bars with the same letter are not significantly different ($p<0.05$) as analyzed using the ANOVA test and then the Tukey's Protected LSD for multiple comparisons in the GLM procedure in SAS.

Discussion

The light intensity treatments had a significant effect on color ratings and biomass, but did not have a significant effect on anthocyanin concentrations of lettuce (Table 1). While the color rating and the biomass were measured for each pot, the anthocyanin determination was measured by combining multiple pots from the same treatment in each block together. This may be one reason the anthocyanin content did not pick up on treatment effects while the color ratings and biomass did. The anthocyanin concentration may be influenced by the water status in lettuce which might be different under different light treatments. In a similar study conducted to test the response of lettuce to light intensity, it was found that foliage dry weight and anthocyanin concentrations were increased by higher light intensity supplied by high-pressure sodium lamps (Voipio & Autio, 1994). This data coincides with our conclusion that the biomass showed significant differences among treatments. However, our anthocyanin data did not show significant differences, therefore, this measurement is inconclusive and should be tested further in future studies.

Light intensity influenced the red coloration of lettuce (Fig. 1 and 2). When lettuce was exposed to the high intensity, the amount of red pigment in the plants was about 2x higher, averaging the two color ratings, compared to the plants exposed to the standard intensity of light. To lettuce producers wanting to grow red lettuce, if in a greenhouse, the use of high intensity light can help improve the amount of red pigment seen in the plant.

If growers are planning on using the high intensity light to increase red pigments, they must introduce plants to the high intensity before plants are almost ready to harvest. When plants were exposed to high intensity light two days before harvest, the rating of red pigments was on average about 2x lower than the high intensity light throughout the growing period. This could be due to the plant not having enough time to adjust to the high light intensity. To improve the 250/500 µmol/m^2/s treatment, the high intensity light could be introduced earlier in the growing season, to allow the plants time to adjust.

Overall the change in intensity caused a lower biomass (Fig. 3). Lettuce producers in a greenhouse environment can use either standard intensity or high intensity light without affecting biomass, but should avoid the sudden swap from standard to high intensity. The standard intensity had a larger biomass accumulation per mol of light provided than the other two treatments. Previous studies show that LED is best to use in an application such as this experiment due to the ability to control spectral composition (Lin et al., 2013). In previously conducted work on tomato plants and light intensities, significant differences in plant biomass were observed (Fan et al., 2013). The highest biomass in tomato plants was under the highest intensity of light, which was consistent with the highest biomass in lettuce under the constant high intensity light in this study. A shortcoming found in this research could be the sudden swap at the two days before harvest in light intensity. There was suddenly a major change in light intensity during this swap that could become a stress for the plants or result in increased transpiration therefore causing the lower biomass in fresh weight. This problem could be corrected by a gradual swap from 250 to 500 µmol/m^2/s over a longer period.

High light intensity at 500 µmol/m^2/s throughout the growing season can lead to higher red pigmentation on leaves and numerically but not significantly higher fresh biomass. This data may be helpful to growers who produce lettuce in a greenhouse setting and are capable of increasing light intensity to improve coloration and maintain productivity available to consumers.

Acknowledgements

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