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HIGH INTENSITY LED LIGHT IN LETTUCE SEED PHYSIOLOGY (*Lactuca sativa* L.)

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Abstract. In order to improve the physiology of plants, this research evaluated the effect of high-intensity LED light (red, blue and green) on the following variables: germination (PG), hypocotyl length (HL), fresh (FW) and dry (DW) weight, in three types of lettuce seed (White Boston, Romana and Black Simpson). Exposure times with colour light were 12, 6 and 3h, with a complement of time for treatments with 6 and 3h of white LED light. We used a completely randomised design with four replications of 30 seeds. Treatments with green and red light to 12h had increases above 90% in HL against the control for the three varieties. The blue light treatment (3h) increased 23% in FW White Boston variety and the red light (3h) increased 14% the DW variable in Roman variety, compared to the control. In this study, treatments with colour light presented results above the control; however, a treatment with a single type of light is not optimal to improve plant physiology. The physiological responses evaluated showed variation related to the genotype of seed and to the time of exposure to high-intensity LED light, so this type of light is a viable option for improving the physiology of plants.

Keywords: LED, high intensity, lettuce, physiology

INTRODUCTION

Light is an important factor for the growth and development of plants. It is a fact that plants are able not only to respond to light intensity but also to its quality or colour (Zhang and Folta, 2012), through their photoreceptors, which are activated under specific wavelengths (Liu 2012, Hogewoning *et al*. 2010). There are three major classes of photoreceptors for plants – phytochromes, cryptochromes and phototropins – which make precise adjustments to their development and growth with respect to different environmental conditions (Chen *et al.* 2004). Cryptochromes are sensitive to blue/UV-A light and responsible for some processes such as the morphology of plants (Lin 2000). Phototropins are responsible for plants orientation to a light source (phototropism) and other responses such as the accumulation of chloroplasts (Zhang and Folta 2012), whose response is stimulated by blue light. The phytochromes, which absorb red and infrared light, are responsible for processes such as germination, reproduction and dormancy (Mathews, 2006). This demonstrates that light is a key factor for plants growth, mainly in controlled environments (Park *et al.* 2012).

In controlled environment agriculture, lighting systems are important (Kozai, 2007) and technological advances in this area are valuable (Bourget 2008). Traditionally, high pressure sodium vapour, fluorescent and incandescent lamps of different spectral emissions have been used for these purposes (Kim *et al.* 2004), but these light sources have limitations, such as short life time, high power consumption and heat emission (Astolfi *et al.* 2012). LED light (light emitting diode) has become an alternative for plant growing systems (Massa *et al.* 2008); these devices are being used as a source of illumination in greenhouses, crop growth chambers and research on growing plants in space (Morrow, 2008; Yeh and Chung 2009, Hogewoning *et al.* 2007, Ilieva *et al.* 2010, Massa *et al.* 2008). The great advantages of this lighting system include the ability to control spectral composition, its small size, the production of high levels of light with a low radiant heat index, and the lifetime of these devices to keep working for years without replacement (Gupta and Jatothu 2013, Xu *et al.* 2012, Bourget 2008) .

Lettuce (*Lactuca sativa*) is one of the most important crops in the world and greenhouse production is one of the most commonly used (Fu *et al.* 2012). Because of the photosensitive characteristics of lettuce seeds, these have been used in research as a model for evaluating their response to the quality of light. Based on the description above, the objective of this study was to evaluate the effect of high light intensity LED type, with different wavelengths (red, blue and green), on the germination and seedling growth of lettuce (*Lactuca sativa*), with different exposure times (3, 6 and 12 h).

MATERIAL AND METHOD

Biological material. Three types of lettuce seed (*Lactuca sativa*) were used, White Boston (1) Roman (2) and Black Simpson (3) varieties, from Itsco, Rancho Los Molinos and Hortaflor brands, respectively, obtained in Mexico City. The seeds were homogenised according to their size, shape and colour, with an average weight (150 seeds) of 0.96, 1.1 and 1.13 mg for White Boston, Romana and Black Simpson, respectively.

LEDs instrumentation. For seed germination, a shelf of 145 × 29.5 × 41 cm was constructed and divided into 10 sections of 14 cm. Shelf walls were made of wood and the edge for the support was aluminium. The interior walls of each section of the chamber were covered with aluminium paper. Nine sections had four LEDs (two colours and two white), plus the section of the control, which had two LEDs (white), located at a height of 24 cm. Three sections were placed with red LEDs (600-650 nm), three blue LEDS (450-500 nm) and three green LEDs (490-540 nm). High intensity LEDs (SILED®) were used, with a power of 5 W, adjusted to an intensity of 550 ± 5 lux, measured with a light meter (Steren®, HER-410 model). Exposure times with colour light were 12, 6 and 3 h, where the complement of time for treatments 6 and 3 h was done with white LED light with 12h photoperiod. To achieve this, a timer card for ignition of the colour and white LEDs was constructed, based on the Microchip PIC16F877 microcontroller, along with a relay system (Fig. 1).

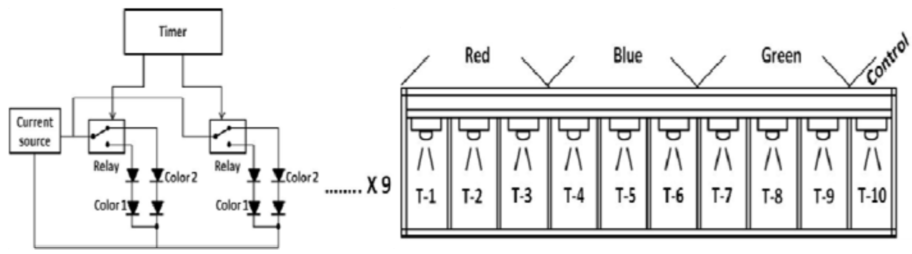

Fig. 1. LED Panel with 10 sections and Timer circuit for timing control

Experimental Design. The experiment consisted of nine treatments of light, the product of three wavelengths (red, blue and green) and three exposure times (3, 6 and 12 h), as well as a control, with white light. The experimental design was completely randomised, with four replications of 30 seeds per experimental unit. The selected seeds were sown and 10 h after, light treatments application began.

Germination test. Planting seeds for germination test was carried out according to the recommendations of ISTA (1999). The seeds were placed in sterile plastic Petri dishes of 5.5 cm diameter, using as the substrate a layer of filter paper moistened with 3 mL of purified water. Each light treatment consisted of 12 Petri dishes, the product of the four replicates for the three varieties of lettuce. Germination took place inside the shelf with alternating cycles of light (12 h) and dark (12 h). Counting of germinated seeds was done at intervals of 12 h in the first four days, then it was performed every 24 h, until the seventh day. Water was added daily to planting.

During the experiment, the following variables were evaluated: 1. Germination velocity (GV). At each count, the germination criterion was the break of the seed and the radical emergence, with length equal to or greater than 2 mm. Germination velocity was calculated, according to that reported by Hussein *et al.* (2011), 2. Percentage of germination (PG). In the final count, lettuce seedlings were considered normal when they had a length equal to or greater than 0.8 cm and had all its parts (root, hypocotyl and cotyledons). Percentage of germination for all treatments was calculated, considering the total number of normal seedlings among the total seeds; 3. Average length of hypocotyl (HL). Hypocotyl length of normal seedlings was measured, for the calculation of average length per treatment, as an indicator of vigour; 4. Fresh weight (FW). Fresh weight (mg) of normal seedlings was determined on a bascule (Velab®, VE-1000 model), and 5. Dry weight (DW). The drying of seedlings was achieved in an electric stove (Riossa®, E-51 model) at a temperature of 65°C for 72 h; once this was finished, dry weight (mg) of seedlings was determined in a bascule (El Crisol®, AR1140 model).

Statistical analysis. Data were subjected to analysis of variance, using the GLM procedure of SAS (SAS Institute, 1998), in a completely randomised design with four replications. Comparison of means was performed using the multiple comparison procedure (LSD), with a significance level of 0.05.

RESULTS

Data analysis showed statistically significant differences in some of lettuce variables assessed. For Variety 1 (White Boston) highly significant differences were shown ($p < 0.01$) between treatments of light (red, blue and green, with three exposure times plus a control) for variables of germination (G24) and speed of germination (V24 and V168) after 24 and 168 h, respectively, as well as for average length of hypocotyl (HL), assessed on the seventh day, whereas for fresh weight (FW) significant differences ($p < 0.05$) were noted between treatments. Variety 2 (Romana) showed highly significant differences ($p < 0.01$) between treatments for HL and dry weight (DW). On the other hand, Variety 3 (Black Simpson) showed differences ($p < 0.1$) between treatments for the final germination percentage (PG) and highly significant differences ($p <0.01$) for HL.

Table 1 presents the comparison of mean values for the variables with statistically significant differences. For variety 1 G24, V24 and V168, all treatments showed similar results, except for the 5th (blue 6 h and 6 h white) and the lowest was 4 (blue 12 h). For the variable HL the best results were obtained for treatments 7 (green 12 h) and 1 (red 12 h), with increases of 159% and 129%, compared with the control, respectively. The FW variable had the highest value for treatment 6 (blue 3 h and 9 h white) followed by 8 (green 6 h and 6 h white), with

Table 1. Comparison of the means for variables measured for 3 varieties of lettuce seed under light treatments

Treatment No.	Time	Light	G24	G168	V24	V168	PG (%)	HL (mm)	FW (mg)	DW (mg)
					1. White Boston					
1	12 h	Red	28a	29.75a	14a	14.52a	97.5a	25.19a	367.5abcd	23.5a
2	6 h	Red	28.25a	29.5a	14.12a	14.41a	90.83ab	17.5b	357.5bcd	22.37a
3	3 h	Red	28a	29.75a	14a	14.38a	95ab	16.23b	345cd	22.85a
4	12 h	Blue	16.5c	29.75a	8.25c	12.34c	97.5[a]	14.46bc	367.5abcd	22.62a
5	6 h	Blue	21.75b	29.75a	10.87b	13.44b	87.5b	11.15c	375abc	22.4a
6	3 h	Blue	28.75a	29.75a	14.37a	14.70a	94.16ab	15.87b	405a	23.42a
7	12 h	Green	29.5a	29.5a	14.75a	14.75a	96.66a	28.44a	322.5d	21.87a
8	6 h	Green	28.5a	29.75a	14.25a	14.6a	98.33a	16.1b	392.5ab	23.2a
9	3 h	Green	28.5a	30a	14.25a	14.65a	96.66a	13.93bc	350cd	21.8a
10	0	White	29.5a	30a	14.75a	14.91a	91.66b	10.98c	327.5d	22.12a
					2. Romana					
1	12 h	Red	28.25ab	29.75a	14.12ab	14.6ab	94.16b	22.98b	362.5ab	24.55ab
2	6 h	Red	28ab	29.75a	14ab	14.5ab	98.33ab	18.43c	310b	25ab
3	3 h	Red	28.25ab	30a	14.12ab	14.59ab	100[a]	18.25c	382.5ab	25.95a
4	12 h	Blue	28.25ab	29.25a	14.12ab	14.45ab	97.5ab	18.19c	357.5ab	23.72bc
5	6 h	Blue	27b	29.75a	13.5b	14.22b	95ab	11.23e	367.5ab	22.55c
6	3 h	Blue	28.25ab	29.5a	14.12ab	14.44ab	96.66ab	18.33c	410a	23.75bc
7	12 h	Green	29.5a	30a	14.75a	14.89a	99.16ab	28.34a	362.5ab	24.17bc
8	6 h	Green	29.25a	29.75a	14.62a	14.72ab	95.83ab	15.65d	345ab	22.6c
9	3 h	Green	29a	30a	14.5a	14.74ab	96.66ab	15.47d	320b	22.62c
10	0	White	28.75ab	29.75a	14.37a	14.66ab	99.16ab	11.97e	357.5ab	22.75c

Table 1. Cont. Comparison of the means for variables measured for 3 varieties of lettuce seed under light treatments

Treatment No.	Time	Light	G24	G168	V24	V168	PG (%)	HL (mm)	FW (mg)	DW (mg)
					3. Black Simpson					
1	12 h	Red	24a	26.75a	12a	12.85a	84.16ab	24.61a	282.5abc	21.82a
2	6 h	Red	22.5ab	27.75a	11.25ab	12.62ab	85.83ab	17.64c	277.5bc	21.92a
3	3 h	Red	21ab	27.25a	10.5ab	12.16ab	83.33ab	17.54c	285abc	21.35a
4	12 h	Blue	23.5a	27.25a	11.75a	12.83a	89.16a	16.05cd	302.5ab	22.7a
5	6 h	Blue	18.25b	26.75a	9.12b	11.37b	73.33c	11.87e	287.5abc	20.77a
6	3 h	Blue	23.5a	26.75a	11.75a	12.74a	85.83ab	16.99cd	315a	21.05a
7	12 h	Green	22.25ab	27.75a	11.12ab	12.66ab	85ab	21.58b	257.5c	20.75a
8	6 h	Green	23a	26.25a	11.5a	12.48ab	88.33ab	16.52cd	275bc	21.07a
9	3 h	Green	20.25ab	26.75a	10.12ab	12.06ab	80bc	15.25d	282.5abc	20.32a
10	0	White	23a	26.25a	11.5a	12.48ab	82.5ab	12.56e	275bc	21.07a

Means with the same letter in a column are statistically equal (LSD, 0.05). G24 = germination at 24 hrs, G168 = germination at 168 hrs, V24 = velocity of germination at 24 hrs, V168 = velocity of germination at 168 hrs, PG = final germination percentage, HL = average length of hypocotyl, FW = fresh weight, DW = dry weight.

increases of 23% and 19% relative to the control, being the lower FW for treatment 7 (green 12 h). Although variables PG and DW did not show significant differences between treatments, those with colour light presented results above those of the control.

On the other hand, for Variety 2 (Romana) mean values of the variables with significant differences, it was observed that for HL, treatments 7 (green 12 h) and 1 (red 12 h) showed increases of 136% and 91%, respectively, against the control, while treatment 5 (blue 6 h and white 6 h) had the shortest length. For variable DW the highest value was shown in treatments 3 (red 3 h and white 9 h), 2 (red 6 h and white 6 h) and 1 (red 12 h), with increases over the control of 14%, 9% and 7%, respectively; conversely, treatment 5 (6 h blue and white 6 h), 8 (green 6h and 6h white) and 9 (green 3 h and 9 h white) obtained lower weight. Although variable FW did not show significant differences between treatments, those with colour light presented results above the control, while for variable PG, control resulted above the light treatments, except for treatment 3 (red 3h and white 9 h).

Also, for variables with significant differences in Variety 3 (Black Simpson), the best results for HL were obtained in treatments 1 (red 12 h) and 7 (green 12 h), with increases of 95% and 71%, respectively, compared with the control, while treatment 5 (blue 6 h and white 6h) had the lowest value of HL. For the variable PG the highest value was obtained in treatment 4 (blue 12 h) with an increase of 8% compared with the control; on the contrary, the lowest value was found in treatment 5 (blue 6 h and white 6 h). Although FW variable did not show significant differences between treatments, those with colour light showed results above the control; while for the DW variable the control value was under the values of red light treatments.

DISCUSSION

The results of this investigation confirm that plant growth can be improved with the use of light sources with specific wavelength, such as high intensity LED light. Performance was evaluated in germination and seed vigour tests of three varieties of lettuce, each of which presented results with significant differences between them, for different variables, despite having the same light exposure treatments. These results suggest that the responses to light quality in lettuce vary with the genotype of the seed (Lin *et al.* 2013, Ohashi-Kaneko *et al.* 2007, Hirai *et al.* 2006). The average length of the hypocotyl was the only variable that showed significant differences in all lettuce varieties used in this research; green light and red light, with 12 h of exposure, were the treatments that achieved the greatest lengths. Other authors have reported that exposure to red light LEDs for 1 week in red lettuce seedlings (*Lactuca sativa* L. cv. Banchu Red Fire) resulted, after 17 days, in a greater height compared to those exposed to blue light (Shoji *et al.* 2010). Also, Kobayashi *et al.* (2013) reported a greater height of miniature lettuce plants grown in hydroponics and exposed to red LED light, which is consistent with the results obtained in this investigation. Other plants, like strawberries (Samouliné *et al.* 2010) and radish (Samouliné *et al.* 2011), in red LED light environments showed similar results, even with other light source such as red fluorescent, in perilla plant (Nishimura *et al.* 2009). This is because phytochrome, photoreceptive proteins responsible for activating some processes in plants, including seed germination, detiolation of seedlings and shade avoidance (Hernández *et al.* 2010), act by monitoring the balance of red and infrared light when they detect changes and respond through the plant photomorphogenesis (Stutte 2009) .

Other studies have shown that treatments with monochromatic red light result in reduction of plant biomass, so it is advisable to supplement them with blue light (Liu 2012, Shin *et al.* 2008, Li *et al.* 2010). In the present investigation, results for the fresh weight variable, although colour light treatments improved in relation to the control (white light), treatment 6 (blue 3 h and white 9 h) was the best; however, the dry weight variable improvement was achieved in treatment 3 (red 3 h and white 9 h) for Variety 2. Other authors have found that red light illumination increases the rate of photosynthesis of the plant, causing an increase in dry weight (Nishimura *et al.* 2009). These findings suggest that exposure to red light produced an increase in DW, but less moisture absorption by the seedling, as the FW was lower compared with seedlings exposed to blue light. It is known that red light promotes seed germination (Jha *et al.* 2010), however, in the present investigation this did not happen.

On the other hand, blue light promoted hypocotyl decrease progressively in treatments with 3 and 6 of exposure. The results agree with those reported by Shoji *et al.* (2010) and Kobayashi *et al.* (2013), where the increase of blue light decreases hypocotyl length of lettuce seedlings, however, 12h irradiation with blue light promoted a slight increase of hypocotyl, in the present study. This decrease is due to cryptochromes (cry) which inhibit stem elongation (Folta and Spalding, 2001). In another aspect, blue light irradiation promotes fresh and dry weight due to the increase of leaves, therefore their effectiveness is mentioned to promote biomass production (Hogewoning *et al.* 2010), consistent with the best treatments for this experiment, where values above those obtained with white light were observed. Final germination percentage (PG) for blue light treatments showed values above the control, in Varieties 1 and 3; the best results were obtained with 12 and 3 h of exposure, respectively, and exposure to 6h of light decreased the PG in all varieties. We would suggest that PG with blue light is a function of exposure time and the variety of seed used.

The results of this research show that green light treatments are consistent with the results of Johkan *et al.* (2012) and McCoshum and Kiss (2011), who found that they promote the growth of plants and seedlings. Other authors have mentioned that green light has an effect on the growth and development of plants (Folta and Maruhnich 2007, Zhang and Folta 2012). In the case of lettuce (*Lactuca sativa*), Kim H. *et al.* (2004) reported that this kind of light penetrates the canopy and potentially increases its development, to promote greater photosynthesis of lower leaves. Green light affects cry receptors and reverses the effects of blue light (Banerjee *et al.* 2007, Folta and Shea 2008). Recent research reports mention the use of green light to enhance the growth of lettuce, in combination with other wavelengths (Kim H. *et al.* 2004, Massa *et al.* 2008), because this light causes excessive stem elongation (Johkan *et al.* 2012), as occurred in this investigation, which resulted in thinning, compared with other treatments. It is also possible to observe that the greater weight (FW and DW) for green light treatment was the treatment of 6 h green light and 6h white light, which is a combination of wavelengths, as recommended by previous authors for improving the growth of lettuce.

In recent years, LED technology has had a spectacular development, with the use of new base materials, such as aluminium indium gallium phosphide (AlInGaP) and indium gallium nitride (InGaN), among others (Yeh and Chung, 2009). This has allowed the development of high intensity LEDs, with powers from 1 W forward, i.e. much higher power than the standard LED. In this study high intensity LEDs of 5 W were used, as an option for growing vegetables and plants in a controlled environment (greenhouse and growth chambers), for the benefits that this technology brings, such as: light intensity; lower energy consumption (energy cost savings of 40%), increased device longevity compared to other lighting systems, increased switching speed, better colour control (Fillipo *et al.* 2010) and being a device which could be environmentally friendly, since it does not use toxic gases, such as fluorescent lamps and mercury (Zhang and Wong 2007), among others.

It is necessary to use technological advances to face social relevant issues such as food production, which has been affected by several factors, among which are the population growth and global climate change (Chakraborty and Newton 2011, Ainsworth and Ort 2010), making it necessary to increase agricultural production (Graham-Rowe 2011). As a result, plant production in controlled environments has grown rapidly around the world to meet food demand (Liu 2012). In this type of agriculture, high intensity LED light could be an alternative to increase the yield.

This suggests a need for further investigation of the effects of treatment with high intensity LED light in different wavelength combinations, and for evaluation of the development of plants, in order to create new lighting systems based on this kind of light for the production of lettuce and other vegetables in controlled environments.

CONCLUSIONS

In the present study we investigated the effect of high intensity LED light of three wavelengths (red, blue and green) in three varieties of lettuce seed (White Boston, Roman, Black Simpson) and the following conclusions were obtained:

1. The seed of the variety Boston showed differences in average hypocotyl length and fresh weight, with increases of 159% and 23%, respectively, in treatments with green light (12 h) and blue light (3 h and white light 9 h) with respect the control (white light 12 h).

2. Roman seed variety had differences in average hypocotyl length and dry weight, with increases of 136% and 14%, respectively, in treatments with green light (12 h) and red light (3 h and white light 9 h) compared to the control (white light 12 h).

3. Black Simpson seed variety showed differences in average length of hypocotyl and final germination, with increases of 95% with red light (12 h) and 8% blue light treatments (12 h), compared to the control (white light 12 h).

4. Physiological responses caused by exposure to different wavelengths of light in lettuce vary with the genotype of seed and with exposure time.

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ZASTOSOWANIE ŚWIATŁA LED WYSOKIEJ INTENSYWNOŚCI W FIZJOLOGII NASION SAŁATY (*Lactuca sativa* L.)

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Streszczenie. W celu uzyskania poprawy fizjologii roślin, w badaniach dokonano oceny wpływu światła LED wysokiej intensywności (czerwone, niebieskie i zielone) na następujące zmienne: kiełkowanie (PG), długość hipokotylu (HL), świeżej (FW) i suchej (DW) masy nasion trzech odmian sałaty (White Boston, Romana i Black Simpson). Czasy naświetlania światłem barwnym wynosiły 12, 6 and 3 h, z uzupełniającym doświetlaniem wariantów z czasami 6 i 3 h białym światłem LED. Zastosowano kompletnie zrandomizowany układ doświadczenia, w czterech powtórzeniach po 30 nasion. Warianty ze światłem zielonym i czerwonym oraz czasami naświetlania do 12 h wykazały ponad 90% wzrost HL w stosunku do kontroli dla trzech odmian. W wariancie ze światłem niebieskim (3 h) uzyskano 23% wzrost parametru FW u odmiany White Boston, a w wariancie ze światłem czerwonym (3 h) 14% wzrost zmiennej DW u odmiany Roman, w porównaniu do kontroli. W badaniach zastosowanie naświetlania światłem barwnym dało lepsze wyniki niż w przypadku kontroli, jednak naświetlanie jednym rodzajem światła nie jest optymalne dla uzyskania poprawy fizjologii roślin. Oceniane reakcje fizjologiczne zmieniały się w zależności od genotypu nasion i czasu naświetlania światłem LED wysokiej intensywności, tak więc zastosowanie tego typu światła stanowi możliwą opcję w poprawie fizjologii roślin.

Słowa kluczowe: LED, wysoka intensywność, sałata, fizjologia