



## Strawberry (*Fragaria ananassa*) seedlings formation under different intensities of violet, blue and red LED light\*

### Formación de plántulas de fresa (*Fragaria ananassa*) bajo diferentes intensidades de luz LED violeta, azul y roja

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## Abstract

**Introduction.** Seedlings formation under controlled conditions is crucial for cultivating species like strawberry. With LED artificial lighting technology allowing greater control over light, variations in components such as wavelength and intensity can be manipulated to generate seedlings with different characteristics. However, there is a need to generate information for the development of precise and efficient light control practices. **Objective.** The evaluate strawberry seedling formation under different intensities of violet, blue, and red LED light. **Material and methods.** The study was conducted in 2020 at the Plant Genetics Laboratory of the Department of Agronomy at the Universidad de Guanajuato, Guanajuato, Mexico. Seeds from San Andreas variety fruits were collected, and seedlings were grown under violet, blue, and red LED light at high, medium, and low intensities. Color determinations of cotyledon area, Chroma saturation index, and Hue angle were performed. Additionally, physio-technical, chemical, and antioxidant activity variables were measured. **Results.** The most suitable treatments were high and medium intensity violet light, as well as high intensity blue light, with germination range exceeding 60 %. Seedlings did not elongate or thin out and exhibited the largest cotyledons areas and chlorophyll concentrations. Furthermore, these light treatments consumed, on average, 31.2 % less electrical energy. **Conclusions.** In addition to white light, the most suitable treatments for strawberry seedlings formation were high and medium intensity violet light, as well as high intensity blue light. It is suggested to evaluate violet and blue light treatments in other cultivars to confirm their positive effect on strawberry seedlings. Red light limited germination and showing higher antioxidant activity.

**Keywords:** agricultural research, chlorophyll, seedlings, seeds.



## Resumen

**Introducción.** La formación de plántulas en condiciones controladas es fundamental para cultivar especies como la fresa. Dado que la tecnología de iluminación artificial LED ha permitido un mayor control de la luz, la variación de componentes como la longitud de onda y la intensidad pueden ser manipulados para generar plántulas con diferentes características. Sin embargo, es necesario generar información para el desarrollo de prácticas precisas y eficientes en el control de la luz. **Objetivo.** Evaluar la formación de plántulas de fresa en diferentes intensidades de luz LED violeta, azul y roja. **Materiales y métodos.** El estudio se realizó durante 2020 en el Laboratorio de Genética Vegetal del Departamento de Agronomía de la Universidad de Guanajuato, Guanajuato, México. Se recuperaron semillas de frutos de la variedad San Andreas y se sembraron plántulas en luz LED violeta, azul y roja a intensidades alta, media y baja. Se realizaron determinaciones de color del área del cotiledón, índice de saturación de color y ángulo Hue. Además, se midieron variables fisiotécnicas, químicas y actividad antioxidante. **Resultados.** Los tratamientos más adecuados fueron luz violeta de intensidades altas y medias; así como luz azul de alta intensidad, debido a que el porcentaje de germinación superó el 60 %. Las plántulas no se alargaron ni adelgazaron, presentaron las mayores áreas de cotiledones y concentraciones de clorofila. Además, estos tratamientos de luz consumieron en promedio 31,2 % menos energía eléctrica. **Conclusiones.** Además de la luz blanca, los tratamientos más adecuados para la formación de plántulas de fresa fueron la luz violeta a alta y media intensidad, así como la luz azul a alta intensidad. Se sugiere evaluar los tratamientos de luz violeta y azul en otros cultivares para confirmar el efecto positivo en las plántulas de fresa. La luz roja limitó la germinación y mostró mayor actividad antioxidante.

**Palabras clave:** investigación agrícola, clorofila, plántulas, semillas.

## Introduction

Plants utilize multiple photoreceptors to perceive various wavelengths, which subsequently trigger physiological responses (Zhang et al., 2018). Plants not only respond to light intensity but also to its duration and quality. These factors and their variations can continuously modify plants physiology, promoting the developed of different mechanisms for constant adjust to varying conditions (Zhang & Folta, 2012).

In strawberry (*Fragaria ananassa*), propagation occurs through stolons, and seeds, depending on the purpose for which the seedlings are required (Ashrafuzzaman et al., 2013; Kang et al., 2020; Zheng, He et al., 2019). Strawberry seeds are germinated in controlled conditions such as humidity, temperature, soil, and recently, artificial light is used to enhance the quality of seedlings formed (Mireles Arriaga et al., 2020; Tewolde et al., 2016). The use of artificial light in seedling production deserves special attention due to the benefits obtained, including disease tolerance, greater vigor, and resistant to handling stress during transplantation (Misu et al., 2018).

Previously utilized light sources included metal halide fluorescent lamps, high-pressure sodium lamps, and incandescent lamps, but Light Emitting Diodes (LEDs) offer several advantages (Samuolienė et al., 2010). LEDs have been employed as an alternative source of artificial lighting due to their low energy consumption, spectral emission control, long durability, reduced size, less heat generation, high plant effectiveness, greater plants proximity, as well as emission of a specific wavelength to improve confined plant cultivation (Singh et al., 2015).

The blue and red lights exert strong effects on plant growth and metabolism and have been utilized to regulate fruit and vegetable quality. Blue light is absorbed by phototropins and cryptochromes, which are involved in plant morphogenesis, stomatal opening, photosynthetic leaf function, high chlorophylls levels generation, leaf expansion, stem elongation, phototropism, transition to flowering. In *Arabidopsis*, proteins involved in phototropism include phot1, phot2, and PKS1 to PKS4, while cryptochromes are represented by proteins cry1 and cry2. Red light affects

flowering initiation, leaf expansion, seed germination, and flowering induction; phytochromes, red, and far-red sensing pigments, are synthesized in an inactive form and convert to an active form after absorbing red light (Costa Galvão & Fankhauser, 2015; Naznin et al., 2016; Zhao et al., 2020). Different combinations of wavelengths have varying beneficial effects on plant development, depend on each species (Olle & Viršile, 2013).

The strawberry crop requires a lower amount of light compared to other horticultural crops such as tomato and melon ( $100\text{-}300\text{ mmol m}^{-2}\text{ s}^{-1}$ ) (Yoshida et al. 2016). Additionally, strawberry plants require less space and can be grow in multiple layers. In Mexico, the strawberry crop covered a planted area of 9,342 ha in 2020, with a total production of 425,007 t, of which 52 % was destined for exportation, mainly to the United States of America (Servicio de Información Agroalimentaria y Pesquera [SIAP], 2021). Seedling production with artificial light treatments could be an alternative to generate vigorous plants. The aim of the present study was to evaluate strawberry seedlings formation under different intensities of violet, blue, and red LED light.

## Materials and methods

### Plant material and location

Seeds were recovered from strawberries (*Fragaria ananassa*) harvested and produced at the same production unit. The San Andreas cultivar has been cultivated since 2004 in the Agrifood region of Guanajuato, Mexico, where the present study was conducted (Wu et al., 2018). The experiments took place in the Plant Genetics Laboratory of the Department of Agronomy at the Universidad de Guanajuato in 2020. Seeds were disinfected using 1 % NaClO solution, after which 100 seeds per test were germinated in transparent humidity chambers measuring  $3,600\text{ cm}^3$  (with dimensions of 15 cm in width, 20 cm in length, and 12 cm in height) filled with distilled water.

### Experimental conditions

The experiment was conducted in a dark growth chamber inside the laboratory with a constant temperature of  $25\text{ }^{\circ}\text{C}$ , which is optimal for germination according to Ito et al. (2011). Additionally, the dark growth chamber had isolated sections for each light treatment. Panels measuring  $1,645\text{ cm}^2$  with a density of 882 SMD5050 RGB LEDs were used as the light source, allowing for intensity and photoperiod control. Blue, violet, and red lights were evaluated as treatments, with white light was included as the control treatment.

Transparent humid chambers (7 L) were positioned 25 cm beneath each panel, with a 12 h photoperiod. The red, green, and blue lights exhibited peaks at 629, 515, and 470 nm, respectively, with intensities measured using the HR 400 UV-VIS spectrum (Ocean Optics). For violet light, the proportions of blue and red were 75.6 % and 25.4 %, respectively. For white light, the proportions of blue, red, and green were 37.4 %, 24.4 %, and 38.2 %, respectively. Three intensity levels were evaluated (high, medium, and low), with the following lux values: blue: 1,036, 643, and 124 lx; violet: 1,080, 671, and 130 lx; red: 495, 312, and 62 lx; white: 1,893, 1183, and 224 lx.

The immediate energy consumption was measured as follows: blue: 2.00, 1.28, and 0.26 A; violet: 2.14, 1.38, and 0.30 A; red: 2.60, 1.68, and 0.34; white: 6.72, 4.30, and 0.86 A.

### Evaluated variables

Germination percentage (GE, %) was measured, with seeds considered germinated when the radicle reached 1 mm in length. Seedlings that surviving 30 days after germination and exhibited developmental potential were

included in a subsequent study. Seedlings were digitized using a 24-bit depth scanner, and root length (LR, cm), stem length (SL, cm), hypocotyl diameter (HD, mm), and cotyledons area (CA, mm<sup>2</sup>) were determined from the digital images. From the CA, values of Lab color space were determined, and subsequently, the Chroma saturation index (*C*) and Hue angle (*H*) were calculated (León et al., 2007). Digital image analysis was performed using an algorithm developed specifically for this study, utilizing the Python 3.7.9 programming language and the Scikit-image 0.18.0 library.

Biomass (BM, mg) was determined by drying the seedlings at 90 °C for 24 h. Total chlorophyll, a, and b concentrations (Chl<sub>a</sub>, Chl<sub>b</sub>, and Chl<sub>a</sub>+Chl<sub>b</sub>, mg mL<sup>-1</sup>) were determined following the method described by Dudek et al. (2014). Proline concentration (PR, μg mL<sup>-1</sup>), as a biochemical stress indicator, was measured following the protocol described by Bates et al. (1973). Phenolic Compounds concentration (PC, μg mL<sup>-1</sup> of gallic acid) was determined using the extraction and measurement procedure outlined by the Martínez-Cruz and Paredes-López (2014). Finally, antioxidant activity was measured using DPPH and ABTS radicals, following the protocols described by Brand-Williams et al. (1995) and Kuskoski et al. (2005), respectively, with the values reported as remaining percentages (%) of each radical.

### Statistical analysis

The data were analyzed using a completely randomized design with five repetitions, with the experimental unit being a commercial container (5X5X3 inches) for fruit containing 100 strawberry seedlings. Tukey mean separation tests ( $\alpha=0.05$ ) were used, and correlation matrix was calculated. Statistical analyses were conducted using Minitab® 16.2.3 software.

## Results

Morphological and physiological measurements subjected to ANOVA, and Tukey (0.05) tests were performed, as showing in Table 1. Highly significant differences were observed in germination percentage (GE) ( $p<0.01$ ). Greater germination percentages were observed under higher intensities of all light treatments. However, the greatest average reduction of 19.2 % was identified under red light.

Highly significant differences were identified in root length (RL) ( $p<0.01$ ), with blue and red lights at their lower intensities being the ones that mostly limited root growth. Compared to white light at its lower intensity, the reductions in RL under blue and red lights were 22.3 % and 33.1 %, respectively. A highly significant positive correlation of 0.83 ( $p<0.01$ ) was identified between RL and GE.

Highly significant differences in stem length (SL) ( $p<0.01$ ) were also identified. There was an increase in SL under blue (2.06 cm), red (2.08 cm), and even violet (2.02 cm) lights at their lower intensities.

Regarding hypocotyl diameter (HD), highly significant differences ( $p<0.01$ ) were observed, with smaller diameters presented at lower intensities under violet, blue, and red lights, measuring 1.20 mm, 1.14 mm, and 1.02 mm, respectively. The greatest average reduction rate of 42.1 % was observed under red light. The respond of SL and HD indicated that low light intensity induced elongation and thinning as seedlings searched for a better light source, eventually leading to the constriction of conducting vessels. This was confirmed by the highly significant negative correlation of -0.80 ( $p<0.01$ ) between both variables.

For biomass (BM) and chlorophyll area (CA), highly significant differences ( $p<0.01$ ) were identified. Higher values were observed under light treatments at high intensities, except for red light and low-intensity blue light. Similar results were identified for BM and CA, which were confirmed by a highly significant positive correlation of 0.85 ( $p<0.01$ ).

**Table 1.** Morphological and physiological variables evaluated in strawberry seedlings (*Fragaria ananassa*) cultivar San Andreas. Plant Genetics Laboratory, Department of Agronomy, Universidad de Guanajuato, Mexico. 2020.**Cuadro 1.** Variables morfológicas y fisiológicas evaluadas en plántulas de fresa (*Fragaria ananassa*) cultivar San Andreas. Laboratorio de Genética Vegetal, Departamento de Agronomía, Universidad de Guanajuato, México. 2020.

Variable	Violet high	Violet medium	Violet low	Blue high	Blue medium	Blue low	Red high	Red medium	Red low	White high	White medium	White low
Germination (%)**	65.2 ±2.77 <sup>a</sup>	62.6± 3.51 <sup>abc</sup>	51.6 ±3.21 <sup>e</sup>	63.8 ±1.79 <sup>a</sup>	60. ±1.79 <sup>abc</sup>	53.0 ±2.12 <sup>de</sup>	63.0 ±3.05 <sup>ab</sup>	57.6 ±3.05 <sup>cd</sup>	44.8 ±3.11 <sup>f</sup>	61.8 ±2.39 <sup>abc</sup>	61.2 ±1.64 <sup>abc</sup>	58.2 ±0.84 <sup>bcd</sup>
Root length (cm)**	3.88 ±0.24 <sup>a</sup>	3.50 ±0.19 <sup>ab</sup>	2.92 ±0.16 <sup>c</sup>	3.56 ±0.11 <sup>ab</sup>	3.56 ±0.25 <sup>ab</sup>	2.44 ±0.33 <sup>d</sup>	3.54 ±0.17 <sup>ab</sup>	3.26 ±0.30 <sup>bc</sup>	2.10 ±0.16 <sup>d</sup>	3.48 ±0.18 <sup>ab</sup>	3.44 ±0.15 <sup>ab</sup>	3.14 ±0.22 <sup>bc</sup>
Stem length (cm)**	1.54 ±0.15 <sup>de</sup>	1.52± 0.11 <sup>de</sup>	2.02 ±0.13 <sup>abc</sup>	1.58 ±0.08 <sup>de</sup>	1.74 ±0.17 <sup>cd</sup>	2.06 ±0.13 <sup>ab</sup>	1.36 ±0.11 <sup>e</sup>	1.78 ±0.2 <sup>abcd</sup>	2.08 ±0.24 <sup>a</sup>	1.76 ±0.17 <sup>bcd</sup>	1.75 ±0.03 <sup>bcd</sup>	1.69 ±0.03 <sup>d</sup>
Hypocotyl (mm)**	1.86 ±0.13 <sup>a</sup>	1.86 ± 0.11 <sup>a</sup>	1.20 ±0.12 <sup>bc</sup>	1.84 ±0.11 <sup>a</sup>	1.40 ±0.14 <sup>b</sup>	1.14 ±0.13 <sup>bc</sup>	2.02 ±0.16 <sup>a</sup>	1.32 ±0.13 <sup>b</sup>	1.02 ±0.11 <sup>c</sup>	1.38 ±0.15 <sup>b</sup>	1.37 ±0.03 <sup>b</sup>	1.37 ±0.03 <sup>b</sup>
Biomass (mg)**	251 ±4.44 <sup>ab</sup>	242 ±5.43 <sup>bc</sup>	202 ±5.63 <sup>d</sup>	252 ±6.75 <sup>ab</sup>	240 ±6.21 <sup>c</sup>	189 ±6.23 <sup>e</sup>	240 ±3.07 <sup>c</sup>	183 ±3.37 <sup>e</sup>	170 ±2.65 <sup>f</sup>	252 ±4.98 <sup>a</sup>	24 ±1.45 <sup>abc</sup>	145 ±1.56 <sup>abc</sup>
Cotyledon area (mm <sup>2</sup> )**	25.9 ±1.06 <sup>a</sup>	20.4 ±1.00 <sup>cd</sup>	17.6 ±1.10 <sup>e</sup>	22.9 ±1.11 <sup>b</sup>	20.2 ±0.84 <sup>cd</sup>	14.0 ±0.94 <sup>f</sup>	18.0 ±1.16 <sup>de</sup>	16.6 ±1.53 <sup>e</sup>	13.2 ±0.90 <sup>f</sup>	23.3 ±1.28 <sup>b</sup>	23.0 ±1.58 <sup>b</sup>	21.2 ±1.30 <sup>bc</sup>
Lightness (L)**	75.2 ±0.78 <sup>a</sup>	73.1 ±1.15 <sup>bcd</sup>	70.2 ±0.69 <sup>fg</sup>	71.2 ±0.89 <sup>def</sup>	68.5 ±1.11 <sup>g</sup>	65.3 ±1.50 <sup>h</sup>	70.5 ±1.20 <sup>efg</sup>	65.5 ±0.51 <sup>h</sup>	64.2 ±0.92 <sup>h</sup>	75.3 ±0.74 <sup>a</sup>	74.4 ±0.28 <sup>abc</sup>	72.4 ±0.16 <sup>cde</sup>
Red/Green value (a)**	-10.1 ±0.28 <sup>a</sup>	-10.0 ±0.33 <sup>a</sup>	-9.8 ±0.33 <sup>a</sup>	-11.0 ±0.30 <sup>bcd</sup>	-11.0 ±0.27 <sup>bcd</sup>	-10.4 ±0.36 <sup>ab</sup>	-11.9 ±0.59 <sup>ef</sup>	-11.2 ±0.51 <sup>cde</sup>	-11.1 ±0.40 <sup>bcd</sup>	-12.1 ±0.26 <sup>f</sup>	-11.8 ±0.23 <sup>def</sup>	-11.6 ±0.25 <sup>cdef</sup>
Blue/Yellow value (b)**	14.2 ±0.48 <sup>cd</sup>	14.2 ±0.56 <sup>cd</sup>	14.6 ±0.23 <sup>c</sup>	16.1 ±0.30 <sup>b</sup>	16.7 ±0.33 <sup>b</sup>	15.0 ±0.27 <sup>c</sup>	12.8 ±0.40 <sup>e</sup>	13.5 ±0.50 <sup>de</sup>	14.2 ±0.46 <sup>cd</sup>	18.1 ±0.36 <sup>a</sup>	18.2 ±0.28 <sup>a</sup>	18.4 ±0.29 <sup>a</sup>
C. saturation index (C)**	152 ±9.25 <sup>c</sup>	152 ±5.20 <sup>c</sup>	155 ±4.64 <sup>c</sup>	190 ±7.78 <sup>b</sup>	200 ±8.39 <sup>b</sup>	167 ±6.93 <sup>c</sup>	153 ±11.55 <sup>c</sup>	153 ±2.68 <sup>c</sup>	163 ±10.06 <sup>c</sup>	237 ±9.18 <sup>a</sup>	235 ±3.76 <sup>a</sup>	236 ±4.65 <sup>a</sup>
Hue angle (H)**	-54.8 ±0.59 <sup>c</sup>	-54.8 ±1.89 <sup>c</sup>	-56.2 ±1.00 <sup>cd</sup>	-55.5 ±0.50 <sup>cd</sup>	-56.7 ±0.25 <sup>cd</sup>	-55.3 ±0.77 <sup>c</sup>	-47.3 ±0.85 <sup>a</sup>	-50.3 ±2.29 <sup>b</sup>	-52.1 ±0.79 <sup>b</sup>	-56.1 ±0.33 <sup>cd</sup>	-57.1 ±0.83 <sup>cd</sup>	-57.8 ±0.85 <sup>d</sup>

Significant differences  $p < 0.05$  (\*), highly significant differences  $p < 0.01$  (\*\*). Values with the same letter within average rows are statically equal according to Tukey ( $p > 0.05$ ). / Diferencias significativas  $p < 0.05$  (\*), diferencias altamente significativas  $p < 0.01$  (\*\*). Valores con la misma letra en los promedios de las filas son estáticamente iguales de acuerdo con Tukey ( $p > 0.05$ ).

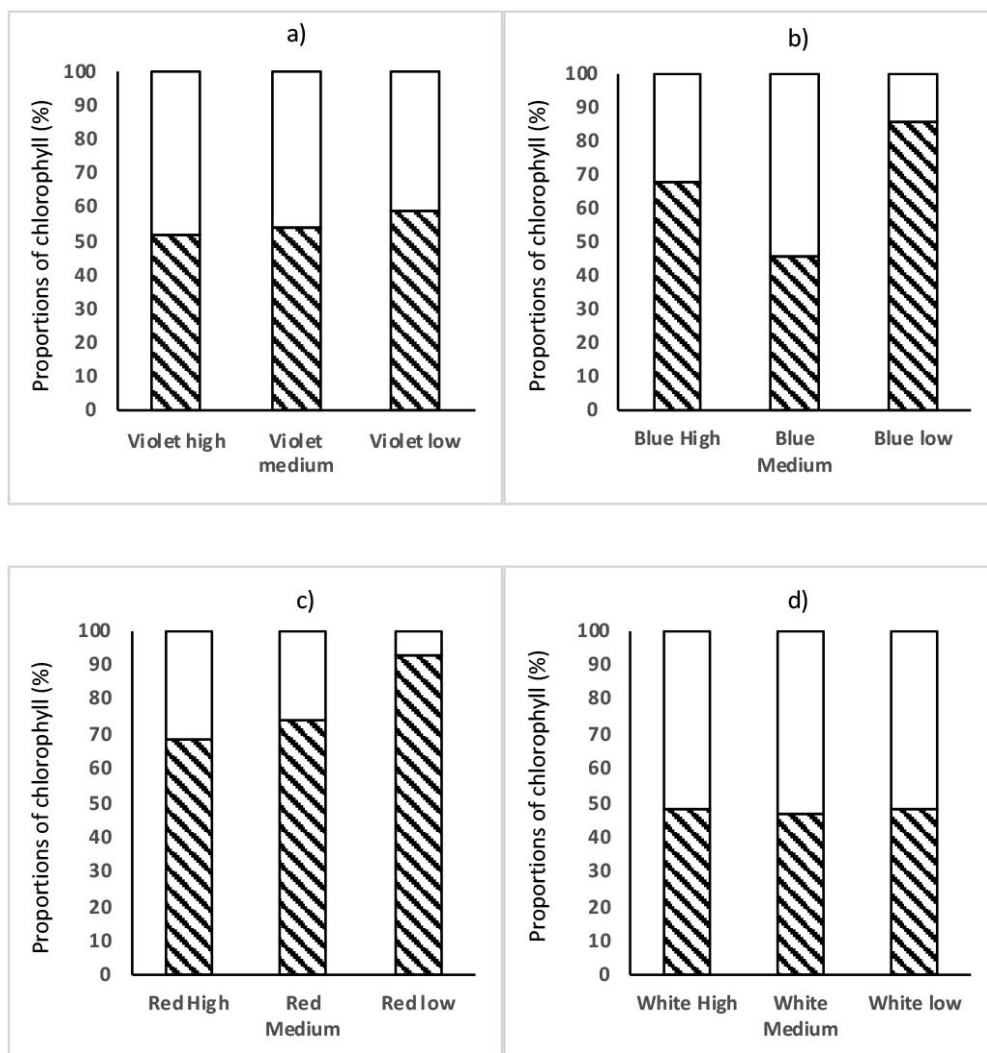
In the case of chlorophyll area (CA) ( $p < 0.01$ ), seedling under the violet light at high intensity presented the highest value of 25.9 mm<sup>2</sup>, followed by white light at high, medium, and low intensities, with 23.3 mm<sup>2</sup>, 23.0 mm<sup>2</sup>, and 21.2 mm<sup>2</sup>, respectively. Under blue light at high intensity, the CA was 22.9 mm<sup>2</sup>; which is noteworthy as this light treatment had lower energy consumption, an important characteristic for larger-scale applications.

For the *Lab* color space components ( $p < 0.01$ ), the highest luminosity (L) values were observed at the highest intensity levels of white, violet, blue, and red light, with values of 75.3, 75.2, 71.2, and 70.5, respectively. Cotyledons under blue and red lights were 7.7 % and 9.9 % less luminous, respectively. In the case of *a* (green/red), all values were negative, particularly under blue and red lights, where values became less negative as light intensity was decreased. Violet light exhibited the least negative values. In *b* (blue to yellow), the highest values were observed under white light, while under red light, there was a tendency for values to increase as light intensity decreased, with an average rate of 7.9 %. This indicated that red light caused cotyledons to turn yellow (etiolated) at higher rate.

Regarding chlorophyll total (Chlt) ( $p < 0.01$ ), the highest concentrations were observed under the highest intensities of white, violet, blue, and red lights, with values of 45.7, 44.9, 40.8, and 39.5 mg mL<sup>-1</sup>, respectively. Each light treatment showed a reduction in Chlt as light intensity decreased, with blue and red lights exhibiting the greatest reduction rates, averaging 41.6 % and 48.7 %, respectively. A highly significant positive correlation of 0.91 ( $p < 0.01$ ) was identified between Chlt and *L*.

Highly significant differences ( $p < 0.01$ ) were identified in chlorophyll a (Chla). The highest concentrations were determined under blue and red lights, with 28.1 and 27.0 mg mL<sup>-1</sup>, respectively. However, under the same light treatments, the greatest reduction rates were observed as light intensity decreased, averaging 48.6 % and 40.3 %, respectively.

In the case of chlorophyll b (Chlb), highly significant differences were identified ( $p < 0.01$ ). The highest concentrations were determined under white and violet lights. The proportions of Chla and Chlb varied depending on the light treatments and their intensities (Figure 1). It should be noted that Chlb variation not only affected Chla values but also the luminosity of the cotyledons area, as highly significant positive correlations were identified between Chlb and Chlt of 0.90 ( $p < 0.01$ ), and between Chlb and *L* of 0.91 ( $p < 0.01$ ).



**Figure 1.** Proportions of chlorophyll a (hatched) and b (white) (%) under a) violet, b) blue, c) red, and d) white lights in strawberry (*Fragaria ananassa*) cultivar San Andreas. Laboratory of Plant Genetic, Department of Agronomy, Universidad de Guanajuato, Mexico, 2020.

**Figura 1.** Proporciones de clorofila a (hatched) y b (white) (%) bajo luces a) violeta, b) azul, c) roja y d) blanca en fresa (*Fragaria ananassa*) cultivar San Andreas. Laboratorio de Genética Vegetal, Departamento de Agronomía, Universidad de Guanajuato, México, 2020.

In the determination of proline concentration (PR) ( $p < 0.01$ ), higher concentrations were observed at lower intensities of violet, blue, and red lights, measuring 3.81, 4.02, and 4.12  $\mu\text{g mL}^{-1}$ , respectively. Notably, for red light at medium intensity, PR was 3.62  $\mu\text{g mL}^{-1}$ , representing the highest proline concentration at this intensity level. Highly significant negative correlations ( $p < 0.01$ ) of PR with GE, RL, CA, L, BM, Chlt, and Chla were identified, with correlation coefficients of -0.78, -0.74, -0.86, -0.83, -0.95, -0.89, and -0.85, respectively.

Regarding antioxidant activity measurement, highly significant differences ( $p < 0.01$ ) were observed in DPPH and ABTS determinations (Table 2). The responses mirrored those seen in chlorophyll concentration, with lower remaining percentages of both radicals observed under medium-intensity red light compared to lower intensity. For DPPH, the values were 53.1 % and 77.1 %, while for ABTS, they were 38.0 % and 57.7 %, respectively. A highly significant positive correlation of 0.97 ( $p < 0.01$ ) was identified between DPPH and ABTS determinations.

**Table 2.** Biochemical variables evaluated in the strawberry seedlings (*Fragaria ananassa*) cultivar San Andreas. Laboratory of Plant Genetics, Department of Agronomy, Universidad de Guanajuato, Mexico. 2020.

**Cuadro 2.** Variables bioquímicas evaluadas en las plántulas de fresa (*Fragaria ananassa*) cultivar San Andreas. Laboratorio de Genética Vegetal, Departamento de Agronomía, Universidad de Guanajuato, México. 2020.

Variable	Violet high	Violet medium	Violet low	Blue high	Blue medium	Blue low	Red high	Red médium	Red low	White high	White médium	White low
Chlorophyll (mg mL <sup>-1</sup> )**	44.9 ± 0.66 a	40.0 ± 0.99 b	32.6 ± 1.01 c	40.8 ± 1.06 b	33.1 ± 1.21 c	14.5 ± 1.40 e	39.5 ± 1.78 b	28.3 ± 1.31 d	12.2 ± 1.11 e	45.7 ± 1.19 a	44.0 ± 0.19 a	40.7 ± 1.76 b
Chlorophyll a (mg mL <sup>-1</sup> )**	22.6 ± 1.75 b	22.3 ± 2.04 bc	20.0 ± 1.16 c	28.1 ± 1.23 a	15.4 ± 0.96 d	13.5 ± 1.29 de	27.0 ± 1.05 a	20.9 ± 0.94 bc	11.3 ± 0.81 e	20.8 ± 1.34 bc	20.7 ± 0.37 bc	19.7 ± 0.30 c
Chlorophyll b (mg mL <sup>-1</sup> )**	21.4 ± 1.07 b	19.2 ± 0.82 cd	14.3 ± 1.53 e	13.4 ± 0.70 e	18.1 ± 1.18 d	3.3 ± 0.68 g	13.7 ± 0.37 e	5.7 ± 0.50 f	1.8 ± 0.55 g	21.9 ± 0.91 ab	23.5 ± 0.48 a	21.0 ± 1.00 bc
Proline concentration (μg mL <sup>-1</sup> )**	0.84 ± 0.10 ef	1.87 ± 0.11 c	3.81 ± 1.13 b	1.00 ± 0.13 e	1.85 ± 0.08 c	4.02 ± 0.07 a	1.34 ± 0.06 d	3.62 ± 0.07 b	4.12 ± 0.06 a	0.78 ± 0.09 f	0.80 ± 0.03 f	0.84 ± 0.04 ef
Phenolic compounds (μg mL <sup>-1</sup> of gallic acid)**	90.2 ± 0.97 e	93.2 ± 1.43 cde	94.7 ± 1.84 bcd	90.1 ± 1.59 e	98.6 ± 0.83 b	96.9 ± 1.02 bc	93.0 ± 1.90 de	106.9 ± 2.37 a	65.4 ± 2.59 f	91.0 ± 2.18 de	91.2 ± 1.78 de	92.8 ± 1.86 de
Antioxidant activity (DPPH, %)**	85.6 ± 1.13 b	80.6 ± 0.95 cd	81.0 ± 1.60 cd	85.6 ± 1.40 b	60.2 ± 1.388 e	58.6 ± 1.85 e	84.2 ± 1.42 bc	53.1 ± 0.98 f	77.1 ± 1.52 d	90.3 ± 1.68 a	90.4 ± 1.28 a	87.4 ± 4.04 b
Antioxidant activity (ABTS, %)**	68.3 ± 1.37 ab	62.3 ± 1.67 d	61.2 ± 1.12 d	63.4 ± 1.44 cd	44.0 ± 1.87 f	41.4 ± 2.05 f	63.0 ± 1.71 cd	38.0 ± 1.76 g	57.7 ± 1.71 e	69.8 ± 1.78 a	68.5 ± 1.12 ab	66.0 ± 1.58 bc

Significant differences  $p < 0.05$  (\*), highly significant differences  $p < 0.01$  (\*\*). Values with the same letter within average rows are statically equal according to Tukey ( $p > 0.05$ ). / Diferencias significativas  $p < 0.05$  (\*), diferencias altamente significativas  $p < 0.01$  (\*\*). Valores con la misma letra en los promedios de las filas son estáticamente iguales de acuerdo con Tukey ( $p > 0.05$ ).

## Discussion

The results of the morphological variables exhibited statistical differences based on the type of light and its intensity. In terms of red light, our findings align with those of Porras Mechán et al. (2020), who observed that seeds treated with red light displayed higher germination percentage, compared to those treated with blue light, as noted in *Campomanesia lineatifolia*. Similarly, Paniagua-Pardo et al. (2015) reported a 25 % increase in germination rates

in *Brassica oleracea* seeds when exposed to high-intensity red light for 12 hours. Photoblastic seeds, which respond to light presence, exhibit enhanced germination, mediated by phytochrome, pigment, or photoreceptor that trigger gene expression changes (Demotes-Mainard et al., 2016).

Contrary to the findings in the present study, Simlat et al. (2016) observed a 26.7 % increase in germination in *Stevia rebaudiana* Beroni seeds exposed to blue light. Interestingly, Hinojosa-Dávalos et al. (2019) found that broccoli seeds treated with blue light had higher germinations rates, whereas those treated with red light exhibited lower rates. Additionally, Jacobsen et al. (2013) reported a significant relationship between coleorhiza and root emergence in *Triticum aestivum* Sunstate seeds after exposure to bright light. Clearly, the wavelength of light plays a pivotal role in germination, albeit with species-specific effects.

The morphological findings in the present study echo those of Spaninks et al. (2020), who investigated *Arabidopsis thaliana* seedlings and the tomato cultivar Moneymaker under red, blue, and white LED lights. Their results demonstrated that red and blue lights induced alterations in root growth, with blue light resulting in the shortest roots length compared to red and white lights. Moreover, Simlat et al. (2016) observed stimulated root elongation in stevia plants with under red light treatment compared to control (white light) at 20 °C, while at 25 °C, treatments of red, white, and white + red light showed no statistically significant differences.

Hypocotyl length responses to different intensities of blue and red light were examined in arugula and mustard plants, revealing those plants exposed to low light intensity displayed longer hypocotyl as part of their shade-avoidance response, without exhibiting etiolation. Similar experiments conducted with *Arabidopsis* and tomato seedlings grown *in vitro* under white, red, and blue lights demonstrated that the highest hypocotyl growth occurred under blue light (Spaninks et al., 2020).

Regarding Cotyledon area and biomass, it appears that red light may not be suitable for obtaining strawberry seedling development. Casierra-Posada et al. (2011) evaluated strawberry stolons under different light conditions and found that the lowest biomass accumulation occurred with red light treatment. Conversely, Zheng, Ji, et al. (2019) observed biomass accumulation in strawberries under high-intensity light. A negative correlation of -0.650 ( $p > 0.01$ ) between photosynthesis rate and maximum efficiency of PSII was observed under a shade conditions, indicating that CO<sub>2</sub> assimilation may be affected by light deficiency, leading to reduce dry matter formation (Choi et al., 2016).

The etiolation effect, characterized by hypocotyl elongation and lack of plastid pigmentation, occurs due to the absence light, as plastid development is only activated in the presence of light by phytochromes (Kusnetsov et al., 2020). Gibberellic acid (GA) plays a crucial role in germination, cell elongation, hypocotyl elongation, and expression of GA2ox and GA3ox genes during germination in many plant species (Çayan et al., 2021). Phytochromes perceive light information, including intensity and duration, and regulate processes such as germination, flowering, and senescence.

Germination processes are regulated by two key growth regulators: abscisic acid (ABA) and gibberellic acid (GA). ABA is associated with seed dormancy under unfavorable conditions, while GA promotes seed germination in favorable environments. However, upon emergence, cotyledons begin to open, initiating chlorophyll biosynthesis, chloroplast development, and autotrophic growth (re-etiolation). Phytochromes play a crucial role in both etiolation and re-etiolation processes (Tripathi et al., 2019). Cryptochromes mediate the blue light regulation of GA levels, influencing GA-induced photomorphogenesis and reducing hypocotyl elongation (Tsuchida-Mayama et al., 2010; Zhao et al., 2020).

The color of cotyledons may have been altered due to the synthesis of different pigments in response to light treatments. This is supported by the highly significant differences ( $p < 0.01$ ) observed in the Chroma (C) and Hue (H) indices (Table 1), which were primarily influenced by variations in *b*. A highly significant negative correlation was identified between C and *b* (-0.77,  $p < 0.01$ ), while a highly significant positive correlation was found between H and *b* (0.98,  $p < 0.01$ ). Colorimetric analyses can be valuable for comparing color values under different conditions and



observing changes in response to LED radiation, aiding in the development of indicators for assessing strawberry health status (García-Noguera et al., 2014).

In experiments with *Rhafanus officinale* plants exposed to red light, higher concentrations of Chlt, Chla and Chlb were reported compared to results obtained in the present study (AbdElgader et al., 2015). Similarly, an experiment with *Lycopersicum* seedlings found higher Chlb content in red light treatment compared to white light. However, contrary to the present study results, these experiment reported higher Chlb concentration in white light than in red light. Additionally, the study found that monochromatic treatment stimulated seed germination at the beginning of the lighting treatment and resulted in taller tomato seedlings with less root biomass (Izzo et al., 2020). In another study, strawberry plants treated with LED lighting exhibited inhibited flower bud initiation due to prolonged light exposure (Hidaka et al., 2014).

Based on these findings, monochromatic red light appears unsuitable for the formation of strawberry, as indicated by the increase in proline concentration (PR), which serves as an indicator of various sources of abiotic stress such as light (Mireles Arriaga et al., 2020). Significant differences ( $p < 0.01$ ) were observed in chlorophyll concentrations, with the most notable variations occurring under red light. Specifically, there was 14.9 % increase between high and medium intensities, followed by a 38.8 % reduction between the medium and low intensities.

The data suggests that under medium-intensity red light, the concentration of phenolic compounds increased in response to light stress, whereas under low intensity, seedlings were unable to maintain the antioxidant response. In contrast, under blue light, there was a 9.4 % increase in chlorophyll between high and medium intensities, with only a 1.6 % reduction under low intensity. This indicates that red light induced higher levels of light stress on the seedlings, while blue light even favored certain morphological and physiological characteristics.

Low light intensity adversely affects dry matter accumulation and reduces plant yield, as plants experience stress due to photon deficiency, resulting in abnormal growth patterns (Banerjee & Roychoudhury, 2016). Conversely, excessive light intensity can also induce stress, leading to severe photoinactivation or photodamage on proteins in the photosystems, such as damage to the D1 protein (PSII) and the large rubisco subunit, as well as a decrease in PSI peptides. High levels of blue light can activate biochemical and physiological processes, resulting in the accumulation of flavonoids in leaves to acclimatize the plant to UV stress (Huché-Thélier et al., 2016).

Studies have shown that the content of antioxidants, such as anthocyanin, in strawberry fruits can be influenced by exposure to different colored plastic films. For instance, Miao et al. (2016) reported higher antioxidant content in fruits exposed to white and red film. Similarly, Manivannan et al. (2017) found that in *Dianthus caryophyllus* plants, red light primarily increased the antioxidant activity of enzymes like superoxide dismutase, guaiac peroxidase, ascorbate peroxidase, and catalase. Additionally, Zhang and Xie (2022) demonstrated that *Brassica rapa* treated with red-violet LED light exhibited delayed postharvest senescence and maintained higher levels of antioxidant enzymes.

However, experiments involving blue-violet light has shown contrasting results. Taulavuori et al. (2018) observed low biomass accumulation in three species but noted a greater accumulation of secondary metabolites compared to the control (Taulavuori et al., 2018). Huang et al. (2020) found that exposure of *Vicia faba* to water stress with violet illumination led to decreased activities of antioxidant enzymes compare to white light, except under high levels of violet light where the effect was similar to white light.

On the other hand, blue light has been shown to promote plant growth, increase nutrient absorption, and enhance crop quality. Wang et al. (2018) demonstrated that different light wavelengths can be used to control the physiology of crops, with blue-violet light promoting protein synthesis followed by carbohydrate synthesis under red-orange light. Similarly, Liu et al. (2022) reported that blue light not only promotes plant growth but also enhances nutrient uptake, yield, and overall crop quality (Liu, et al. 2022).

## Conclusion

Aside from white light, the most effective treatments for strawberry seedlings formation were violet light at high and medium intensities, along with blue light at high intensity. Regardless of treatment, the germination rate was 60 % under high light intensities; without these treatments, it averaged 51.9 %. Under these conditions, seedlings did not elongate or thin out, had cotyledons with the largest surface area, and exhibited the highest chlorophyll concentrations. The energy consumption of these treatments was, on average, 31.2 % lower than that of their corresponding white light treatments. In contrast, red light was unsuitable for seedling formation due to its high induction of light stress.

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