Article

Use of different proportions of red and blue LEDs to improve the growth of *Lilium* spp.

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Abstract

The combination of red and blue light emitting diodes (LEDs) is an effective light source for plant growth and development. Several species of the genus *Lilium* are valued as cut flowers, but the information on the effect of the quality of light on their growth is still very scarce and it is necessary to determine the optimal light spectrum that allows obtaining desirable characteristics. The objective of this paper was to evaluate the growth of *Lilium* spp. 'Corvara' with LED lighting. The proportion of red and blue LED lights in each treatment was: 20:80 (R4B); 40:60 (2R3B); 60:40 (3R2B); 80:20 (4RB) and the control (W) with 100% white light. All treatments had a photosynthetically active radiation of 90 ±10 μmol m^{-2} s^{-1} during a photoperiod of 14 h. The work was conducted in Chapingo, Mexico in 2019 and 2020. The results showed that, the lowest number of days to harvest maturity (91 days) and the plants of lower height (83 cm) occurred with the illumination of treatment 2R3B. Regarding the control, increases were achieved in the following variables: with treatment R4B, leaf area 26% and tepal color 13% in the variable Chroma; with 3R2B, vase life 6%, with 4RB, plant height 21%, flower diameter 5% and the number of days to maturity was 16. It is concluded that the proportion of red and blue light modifies the growth and development of *Lilium* spp. 'Corvara' plants.

Keywords: cut flowers, LED's, supplemental lighting, vase life.

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Introduction

Worldwide, *Lilium* crop ranks eleventh in demand within ornamentals, which is attributed to the diversity of colors and the availability of the flower throughout the year (García and Companioni, 2018). Statistics of the Agrifood and Fisheries Information Service (SIAP, 2020) indicate that in Mexico in 270 ha, a production of 735 472 gross was reached.

The response of plants to the received light spectrum is determined by the action of the various photoreceptors, according to Xie *et al*. (2019), these can be grouped according to the region of the electromagnetic spectrum they detect: phytochromes detect red (600 to 700 nm) and far red (700 to 750 nm) in a dynamic photo equilibrium ratio and cryptochromes and phototropins respond to blue light from 350 to 500 nm (Fantini *et al*., 2019).

Light emitting diodes (LEDs) are the first light source that allows the selection of specific wavelengths in the lighting spectrum that coincide with the absorbance of plant photoreceptors and impacts specific vital processes (Morrow, 2008). The number of light hours per day directly impacts the flowering of plants; in this aspect, plants can be divided into three categories according to the length of the day that is required to trigger flowering: short-day plants, day-neutral plants and long-day plants. *Lilium* is classified as a facultative long-day plant; that is, it can flower under a wide range of day length, but flowering is accelerated or increased over long days (Dole and Wilkins, 2005).

Lighting with LEDs has a wide potential for the cultivation of ornamental plants; it has been used as a supplemental light source for propagating seedlings and cuttings of calibrachoa (*Calibrachoa hibrida*), in which the development of roots and shoots was higher with a combination of white and blue LEDs (Olschowski *et al*., 2016), it is a non-polluting form to control plant height (Bergstrand *et al*., 2016), the extension of the stem can be reduced and result in more compact plants in impatiens (*Impatiens walleriana*), petunia (*Petunia hibrida*) and sage (*Salvia officinalis*) with a higher proportion of blue light that complements red light (Wollaeger and Runkle, 2013), in celosia plants (*Celosia argentea*), impatiens, petunia, calendula (*Calendula officinalis*), sage and pansy (*Viola odorata*) cultivated under proportions of light red: blue of 85:15 and 70:30.

More compact plants were obtained, with a larger stem diameter and a higher chlorophyll content than plants grown with high-pressure sodium lamps (Randall and López, 2014), far-red LED lights inhibit the flowering of chrysanthemum (*Chrysanthemum morifolium*) and promote it in snapdragon (*Antirrhinum majus*) and petunia, while with high intensities of blue light, flowering is induced in coreopsis (*Coreopsis grandiflora*) and rudbeckia (*Rudbeckia hirta* L.), this allows growers to control flowering according to market demand (Meng and Runkle, 2016).

The specific responses of plants to the light spectrum can sometimes be predictable based on published research; however, the general reaction of plants is difficult to predict due to the complicated interaction of many different internal responses, caused by the action of different photoreceptors (Wollaeger and Runkle, 2013), which have an effect on primary (production of amino acids, nucleotides, sugars and lipids) and secondary (terpenes, phenols and alkaloids) metabolism (Darko *et al*., 2014).

The combination of red and blue LEDs is an effective light source for plant growth and development; however, the response to light quality varies between species and even between cultivars and their stage of development (Bayat *et al*., 2018). The objective of this work was to evaluate the effect of LED lighting on the cultivation of *Lilium* spp. 'Corvara' to find the proportion of LED light that allows having desirable characteristics and greater control of growth, flowering and vase life with lighting provided by LED lamps with different proportions of blue light (B), red light (R) and white light (W) as a witness, the hypothesis is that only with the combination of red and blue light, it is possible to have normal growth.

Materials and methods

Localization

The study was conducted in a growth room made of wood built inside a glass-covered greenhouse of the Horticulture Institute of the Chapingo Autonomous University, located at an altitude of 2 240 m and at 19° 29' north latitude and 38° 53' west longitude, National Meteorological Service (2021). The growth room was divided into five compartments with an area of 0.91 m^2 each. Relative humidity and ambient temperature (Table 1) inside the growth room were monitored with a Perfect Prime® data logger (Japan).

Table 1. Temperature and relative humidity inside the growth room during the cultivation cycle of *Lilium* spp 'Corvara' with LED supplemental lighting.

Month	Diurnal temperature (°C)		Nocturnal temperature (°C)		Relative humidity (%)	
	Min	Max	Min	Max	Min	Max
October	22.8	27.2	12	19.6	38.5	65.4
November	19.1	24.5	9.1	16.8	45.4	68.9
December	13	23.4	7.4	14.1	44	70.1
January	11.6	23.3	6.9	13	42.1	58.9
February	17.7	24.8	9	14.3	46.5	67.6

Plant material and substrate

Bulbs of 20 to 22 cm perimeter of *Lilium* spp. 'Corvara' of the oriental type (flower with pink tepals with white border) were used, the bulbs were washed with water, disinfected with Tecto® (2 g L^{-1}) and placed 7 cm deep from the apex of the bulb, in black plastic bags of 8 L, on October 29, 2019. Red tezontle with a particle diameter of 2 to 3 mm was used as a substrate, due to its availability, low acquisition cost, water retention capacity and neutral pH (Ojodeagua *et al*., 2008).

Nutrient solution

The Steiner solution (1961) was used at 75% (NO_3^-, $H_2PO_4^-$, SO_4, K^+, Ca^{2+} and Mg^{2+}, with a concentration of 9, 1, 5, 5, 7 and 3 meq L^{-1}, respectively) throughout the culture cycle. To supply the micronutrients, 0.025 g L^{-1} of Tradecorp® was used, which contains a mixture of fertilizers

chelated with EDTA, which contains: iron 7.5%, manganese 3.5%, zinc 0.7%, boron 0.65%, copper 0.28% and molybdenum 0.26%. The nutrient solution was prepared with drinking water considering the chemical properties for its formulation. The pH of the nutrient solution was adjusted to 5.7 with sulfuric acid; the electrical conductivity was 2.1 dS m^{-1}. Each plant was watered every day manually with 200 mL the first 15 days, then 350 mL was applied.

Treatments

A treatment was located in each compartment of the growing room, each one consisted of 200 Watts lamps, composed of 20 TIANLAI® (China) monochromatic LEDs. The proportion in % of red and blue LED lights in each treatment was: 20:80 (R4B); 40:60 (2R3B); 60:40 (3R2B); 80:20 (4RB) and the control (W) with 100% white light. The lamps were raised as the plants grew so that light would affect the apexes with a photosynthetically active radiation (PAR) of 90 ±10 mmol m^{-2} s^{-1}, the PAR was determined with a hidrofarm® quantum meter (CA, USA).

The wavelengths of the LEDs were: blue from 450 to 480 nm with a maximum at 465 nm, red from 620 to 650 nm with a maximum at 635 nm and white with a light spectrum from 400 to 700 nm, which were within the range indicated by Deram *et al*. (2014), who pointed out that the combination of blue light (425-490 nm) and red light (610-700 nm) are the best light spectra for plant photosynthesis. The wavelengths emitted by the LEDs were measured with a spectroradiometer (CS-2000, Konica Minolta®, Japan). The lamps were kept on continuously from 7 to 21 h, the on and off was programmed with a Voltech TEM-8 digital timer (China).

Experimental design

It was a completely randomized design, five treatments and nine repetitions, the experimental unit consisted of one plant per bag, with a total of 45 experimental units.

Variables evaluated

The flower stems were cut from January 29 to February 12, 2020, when the first flower of each plant was fully open. From each treatment, the first four plants were taken to evaluate vase life, each stem was cut the leaves except those of the inflorescence and from those leaves, samples were taken to determine the concentration of total soluble sugars and photosynthetic pigments, for this reason, the variables mentioned have four repetitions. In the remaining five plants of each treatment, the leaf area and stomatal density were evaluated, as described later. The other variables were measured in nine repetitions per treatment. The determination of the evaluated variables is described below.

Days to harvest maturity and duration of the vegetative stage (days)

The days to maturity were counted from the planting of the bulbs to the cutting of the flower stems; the vegetative stage was counted from the planting of the bulbs until the first floral bud was visible on each plant.

Plant height (cm)

It was measured with a tape measure from the base of the stem to the apex of the floral buds.

Fresh and dry weight of the aerial part (g)

The plant was separated in aerial part, bulb and roots, the fresh weight was recorded with an Ohaus® digital scale Scout Pro model (NJ, USA), then the samples were washed with distilled water, dried in an oven with circulating air Binder® (Berlin, Germany) at 65 °C for 48 h and the samples were weighed again.

Stem diameter (mm)

It was measured under the inflorescence with a Trupper® digital vernier (China).

Leaf area (cm^2)

All the leaves were separated from the stem, except the leaves of the inflorescence, it was evaluated with a Li-cor 3100 leaf area integrator (NB, USA).

Flower diameter (cm)

It was measured with tape measure between the apexes of the petals of the first fully open flower of each plant.

Number, length (cm) and diameter of buds (mm)

The floral buds of each plant were counted when cutting the flower stems; the length of the first closed bud that presented the pink color of the cultivar was measured in each plant, from its base to the apical part, in the same bud, the diameter in the widest part was measured with a Trupper® digital vernier (China).

Color (L, H, C)

It was determined in three different points of the middle part of the tepals of the first flower opened in each plant, with a X- Rite Inc. spectrophotometer (MI, USA), the parameters were obtained: L which is brightness or luminosity, whose values range from 0 to 100, where 0 represents the black color and 100 the white, H or hue, which is defined as the angle between the hypotenuse and 0° with the axis (blue green to purple red), C or color purity (chroma), which reports the saturation index, these values form the chromatic model used to describe all the colors that the human eye can perceive (Jakopic *et al*., 2007).

Stomatal density (stomatal mm^{-2})

On one leaf per plant, on the underside of basal leaves (15 cm high from the base of the stem), the middle part (35 cm high) and apical parts(60 cm high), two layers of transparent varnish were applied in five locations next to the midrib of each leaf, they were left to dry, the plants were cut

and the layers of varnish with the epidermis (negative of the impression) were removed from the leaves, placed on slides and with a Carl Zeiss® optical microscope (Germany), the number of stomata in five visual fields (10x) was counted per each slide.

Photosynthetic pigments (mg g $^{-1}$ fresh weight)

The concentrations of chlorophyll a, b, total chlorophyll and carotenoids were determined when flowering began, from 36 to 46 days after planting and after cutting the flower stems as indicated by the AOAC (1980) and the technique described by Witham *et al*. (1971), for which two newly matured leaves were used per plant. Absorbance was read at 663, 645 and 470 nm on a Thermo Spectronic ® spectrophotometer, Genesys model 10 UV (WI, USA).

Total soluble sugars (mg g^{-1} fresh weight)

They were evaluated in leaves at the beginning of flowering and in leaves, bulbs and roots at the time of cutting the flower stems, with the anthrone method described by Witham *et al*. (1971), 2 g of the mixture of three leaves of recent maturation, 2 g of the scales of the bulbs and 2 g of the middle part of the roots were weighed. The readings were made at 600 nm with a Thermo Spectronic® spectrophotometer, Genesys 10 UV model (WI, USA). The concentration of sugars was calculated from a pattern curve containing up to 250 mg of glucose ml^{-1}.

Vase life (days) and water consumption in postharvest

It was counted from the cutting of the plants until 50% of the flowers of each stem presented symptoms of senescence. The flower stems were left at 70 cm in height, cutting the base diagonally and the lower foliage was removed; each stem was placed in a test tube of 1 L capacity with 500 mL of running water, there was an average temperature of 18.5 °C, the average relative humidity was 41.7%. The consumption of water by evaporation and perspiration was determined every day in each container by difference between the initial volume and the final volume, replenishing the water consumed.

Data analysis

An analysis of variance, Tukey's comparison of means ($p< 0.05$) and Pearson's correlation analysis were performed with the statistical program Statistical Analysis System (SAS) version 9 (SAS Institute Inc., 2002). The graphs were made in the Sigmaplot program.

Results and discussion

Growth and flowering variables

The plants of oriental *Lilium* 'Corvara' showed significant differences between treatments ($p< 0.05$) in the variables days to harvest maturity, duration of the vegetative stage, plant height, stem diameter, fresh and dry weights of the aerial part, flower diameter and bud length, treatment 4RB (80% red light + 20% blue light) registered the maximum values (Tables 2 and 3). Although this treatment favored the elongation of the internodes, it increased the duration of the cultivation cycle by 15 days with respect to the control.

Table 2. Effect of red, blue, white LED supplemental light and its combinations on the growth variables of *Lilium* spp. 'Corvara'.

Treatment	Days to harvest maturity (days)	Duration of the vegetative stage (days)	Height (cm)	Stem diameter (mm)	Fresh weight of the aerial part (g)	Dry weight of the aerial part (g)	Leaf area per plant (cm^2)
R4B	100.2 b	35.9 c	88.1 b	9.4 b	199.6 ab	25.7 ab	1161 a
2R3B	90.9 d	38.2 b	83.2 b	9.5 b	166.3 b	22.1 b	1045 ab
3R2B	97.7 c	36.2 bc	85.2 b	9.5 b	196.3 ab	22.8 b	1044 ab
4RB	106.9 a	46 a	101 a	10.5 a	238.6 a	28.6 a	1046 ab
W	91.4 d	37.9 bc	83.4 b	9.7 ab	216.5 ab	24 b	907 b
CV (%)	1.5	4	8.6	6.9	19.1	11.8	7.5
DSH (0.05)	2	2.1	10.3	0.9	52.2	3.9	148

Values in the same column followed by the same letter are not statistically different (Tukey, $p \leq 0.05$); CV= coefficient of variation; DSH= honestly significant difference. R= red light; B= blue; and W= white; R4B= R 20% + B 80%, 2R3B: R 40% + B 60%, 3R2B: R 60% + B 40%, 4RB: R 80% + B 20% and W= 100% white light.

Table 3. Effect of red, blue, white LED supplemental light and their combinations on flowering variables of *Lillium* spp. 'Corvara' plants.

Treatment	Flower diameter (cm)	Number of buds	Bud length (cm)	Bud diameter (mm)	Color of tepals		
					L	C	H
R4B	28.4 ab	5.8 ns	14.3 ab	41.5 ns	51.7 c	24.3 a	348.8 ns
2R3B	25.3 b	5.5	12.8 b	38.9	55.2 bc	23.7 a	349.4
3R2B	28.1 ab	4.6	14.5 a	42.9	57.4 b	21.7 ab	352.7
4RB	29.9 a	5.8	14.6 a	38	66.3 a	16.2 b	349.5
W	28.5 ab	5.7	14.8 a	42.3	55.4 bc	21.5 ab	347.6
CV (%)	9	19.4	8.1	9.5	7.3	24.2	1.8
DSH (0.05)	3.4	1.43	1.5	5.2	5.6	7	8.4

Values in the same column followed by the same letter are not statistically different (Tukey, $p \leq 0.05$); CV= coefficient of variation; DSH= honestly significant difference; ns= not significant; L= brightness or luminosity; C= chroma or purity of color; H= hue. R= red light; B= blue; and W= white; R4B= R 20% + B 80%, 2R3B: R 40% + B 60%, 3R2B: R 60% + B 40%, 4RB: R 80% + B 20% and W: 100% white light.

At the time of cutting, the average height of the stems of treatment 4RB was 101 cm, this is important since the quality standards for the commercialization of this cut flower are based on the length of the stems and the number of buds, an example is Coxflor® (a company that supplies bulbs for flower production), it marks a quality classification for the commercialization of *Lilium*: category plus 4 to 6 buds per stem and a height of 90-110 cm; exp (export), 2 to 3 buds per stem with a height of 70 to 90 cm and med (average) from 1 to 2 buds per stem with a height of 70 cm.

Based on the above, only plants grown with treatment 4RB would enter the plus category, the others in the exp category. The plants of lower height (83 cm) were obtained with the control and treatment 2R3B.

Bergstrand *et al*. (2016) reported that stem elongation is a response of phytochrome, which is concentrated in apical meristems that are regions where drastic changes in development occur, a lighting supplemented with red light increases the height of the chrysanthemum which as *Lilium* are long-day plants, but the effect is opposite in poinsettia plants (*Euphorbia pulcherrima*), which is short-day plant (Dole and Wilkins, 2005), in which the height is reduced. The increase in fresh and dry weight of the plants under treatment 4RB also corresponded with a larger stem and flower diameter. These results are consistent with what Chen *et al*. (2018) report, who claimed that the highest dry weight is obtained under red light.

The maximum value of leaf area was recorded in treatment R4B (treatment with 20% red light and 80% blue light), which is in accordance with what was obtained by Chen *et al*. (2018), who found that the receptors of blue light (cryptochromes and phototropins) have an effect on the development of leaf area. As for the colorimetric parameters, it is observed that the value of C increased with the proportion of blue light, which resulted in tepals of a more intense pink color; on the contrary, the highest value of the L (ranging from 0 for black color to 100 for white color) occurred in 4RB causing paler tepals.

The color of the flowers is one of the most important characteristics in ornamental plants, as it affects their commercial value, the colors of *Lilium* flowers are derived mainly from anthocyanins and carotenoids, anthocyanins are the main pigment in cultivars with pink and brown flowers while carotenoids are derived from cultivars with yellow and orange flowers, both anthocyanins and carotenoids contribute to the red color (Kong *et al*., 2015).

There is only one anthocyanin, cyanidin, in the petals of various colors of flowers ranging from light pink, pink, dark red and brown, but the content is different; the difference in tone is positively correlated with the content of anthocyanins (Yamagishi *et al*., 2012). In rose (*Rosa hibrida*), chrysanthemum and campanula (*Campanula portenschlagiana*), Ouzounis *et al*. (2014) found that a high proportion of blue light increased the concentration of anthocyanins.

Stomatal density

The maximum value was recorded in basal leaves of R4B (Figure 1), where the fluency rate (number of absorbed photons per unit area) was lower (35 mmol m^{-2} s^{-1}) than in the apexes of the plants (90 mmol m^{-2} s^{-1}). According to Matthews *et al*. (2020), the stomatal aperture is driven by two different routes: with red light, there is the mechanism that coordinates stomatal behavior with photosynthesis and occurs at high fluency rates; the specific response to blue light is saturated at low intensities (5 to 10 mmol m^{-2} s^{-1}), it is independent of photosynthesis and causes stomatal aperture in the mornings when the solar spectrum is enriched with wavelengths of the blue.

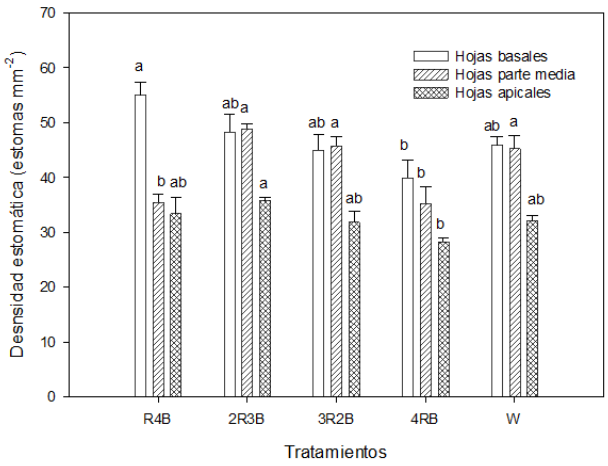

Figure 1. Effect of red, blue, white LED supplemental light and their combinations on stomatal density in *Lillium* spp. 'Corvara' leaves. Means with the same letters are no different (Tukey, $p \leq 0.05$); R: red light, B: blue and W: white; R4B: R 20% + B 80%, 2R3B: R 40% + B 60%, 3R2B: R 60% + B 40%, 4RB: R 80% + B 20% and W: 100% white light.

Pigments

Figure 2 shows that the highest concentration of chlorophyll a, b, total and carotenoids before flowering and at the time of cutting the stems was obtained in treatments with the highest percentage of red light (4RB and 3R2B).

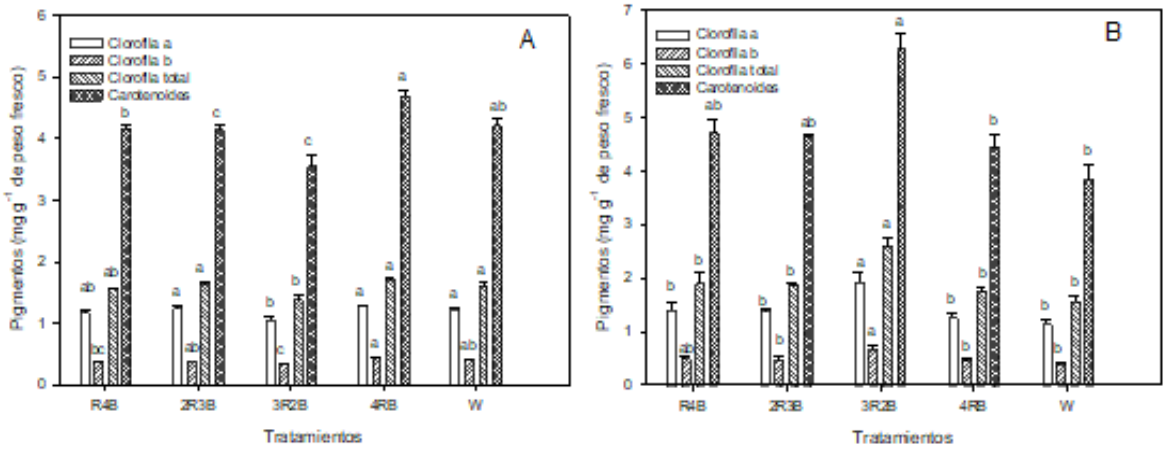

Figure 2. Effect of red, blue and white LED supplemental light and their combinations on the foliar concentration of pigments: A) before flowering; and B) after the cutting of *Lillium* spp. 'Corvara'. Means with the same letters are not different between pigments (Tukey, $p \leq 0.05$); R: red light, B: blue and W: white; R4B: R 20% + B 80%, 2R3B: R 40% + B 60%, 3R2B: R 60% + B 40%, 4RB: R 80% + B 20% and W: 100% white light.

Folta and Childers (2008) indicated that, although chlorophylls a and b have their absorption peaks in the range of blue and red of the spectrum, the absorption effect of the different photoreceptors overlaps, in addition to the fact that under high light conditions, the phytochromes, cryptochromes and phototropins are saturated, so it is difficult to differentiate the scope of each of them. With

respect to the other pigments, the concentration of carotenoids was higher, which with an absorption spectrum between 350 and 500 nm efficiently capture much of the light not absorbed by chlorophylls a and b for photosynthesis (Ouzounis *et al*., 2015). In rose (*Rosa hibrida*), it was found that the quantity of carotenoids decreased in plants grown under blue light (Bayat *et al*., 2018), which is consistent with the results of this work.

Total soluble sugars

In all treatments, the concentration of sugars in the leaves decreased during flowering, at the time of harvest, they were concentrated in the bulb with respect to the other organs (leaves and roots), since the scales of the bulb being modified leaves store water and reserve substances (Dole and Wilkins, 2005).

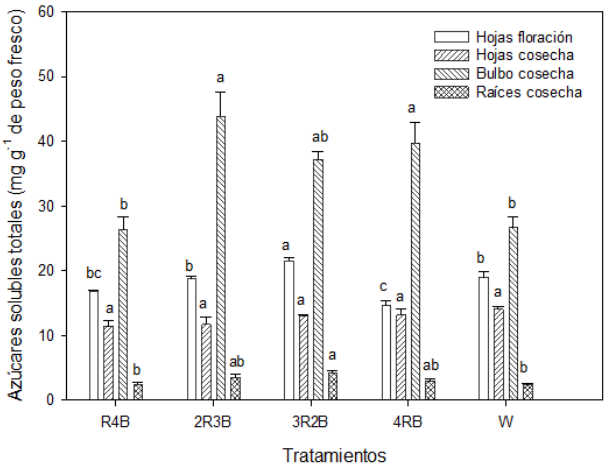

Figure 3. Effect of red, blue, white LED supplemental light and their combinations on the concentration of total soluble sugars of *Lilium* spp. 'Corvara'. Means with the same letters are not different between organs (Tukey, $p \leq 0.05$); R: red light, B: blue and W: white; R4B: R 20% + B 80%, 2R3B: R 40% + B 60%, 3R2B: R 60% + B 40%, 4RB: R 80% + B 20% and W: 100% white light.

In all treatments, the concentration decreases significantly during flowering because the demand for photosynthates is accentuated when the floral bud. At the time of harvest, the sugars are concentrated in the bulb with respect to the other organs, since the scales of the bulb being modified leaves store water and reserve substances (Dole and Wilkins, 2005).

Vase life and water consumption in postharvest

The plants under treatment 3R2B accumulated more sugars in the leaves for the flowering period (Figure 3), which could be available until the postharvest, so they had a longer vase life, which is an advantage for their commercialization, although statistically it is not different from the vase life that was obtained with treatment 2R3B. In lisianthus (*Eustoma grandiflorum*), the sugar content favored the development of the bud and the flower opening, in addition to increasing vase life (Cruz-Crespo *et al*., 2006). The plants with the longest vase life were the ones that consumed the most water in postharvest (Figure 4).

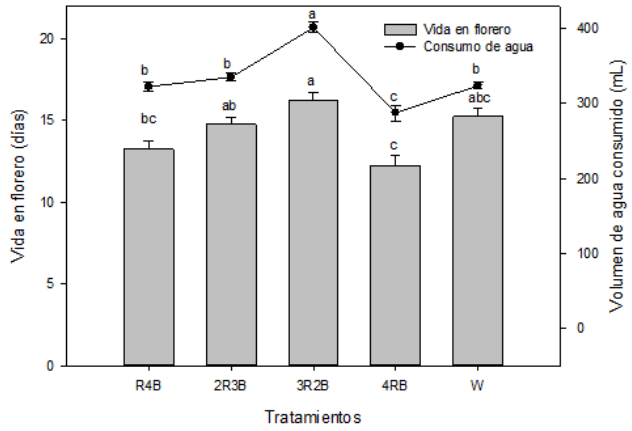

Figure 4. Effect of red, blue, white LED supplemental light and their combinations on vase life and water consumption in postharvest in *Lilium* spp. 'Corvara' plants. Means with the same letters are not different between organs (Tukey, $p \leq 0.05$); R: red light, B: blue and W: white; R4B: R 20% + B 80%, 2R3B: R 40% + B 60%, 3R2B: R 60% + B 40%, 4RB: R 80% + B 20% and W: 100% white light.

There was no fall or abortion of floral buds, no pests or diseases were detected in any of the treatments, contrary to what was found in tomato plants grown without blue light, in which foliar disorders developed (Wollaeger and Runkle, 2013); as well, in lisianthus, the luminous intensity affects the quality of the flowers, low luminous radiation causes abortion of flowers, yellowish leaves and discoloration of petals (Griesbach, 1992).

Pearson linear correlation

They were detected between the variable's height *vs* dry weight of the aerial part (0.89), height vs. days to harvest maturity (0.74) and dry weight of the aerial part vs. flower diameter (0.87), so there is a positive relationship between the variables dry weight of the aerial part, height and diameter of the flower.

Conclusions

The different proportions of red and blue light modified the growth and development of *Lillium* spp. 'Corvara'. The treatment with 20% red light + 80% blue light favored the development of the leaf area and the most intense petal color, the shortest growing cycle was obtained by applying 40% red light + 60% blue light; the longest vase life occurred in plants grown with 60% red light + 40% blue, while, with 80% red light + 20% blue light, the flowers of largest diameter and the tallest plants were obtained.

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