

Impact of light quality on the physiological characteristics of *Capsicum chinense* seeds

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: The objective of this work was to evaluate the physiological quality of *Capsicum chinense* seeds submitted to different light spectral qualities. It was used a completely randomized design, in a 4x5 factorial scheme, with four pepper cultivars [BRS moema biquinho yellow (Biq. Yellow), Airetama biquinho red (Biq. Red), Boyra Habanero red (Boyra Hab. Red), BRS Seriema tupã bode red (Tupã Bode Red)] and five light spectral qualities, being blue LEDs (B-LEDs); red LEDs (R-LEDs); blue+red LEDs (BR-LEDs); white LEDs (W-LEDs) and fluorescent lamp (FL) carried out germination and vigor analysis, with four replicates of 50 seeds. For this, the seeds were conditioned inside gerbox® boxes and kept in a growth room. The Biq. Yellow and Boyra Hab. Red peppers showed the highest potential of germination and vigor, respectively, indicating high physiological quality. In general, the light spectral qualities provide differentiated responses in the initial development of the pepper cultivars, being the reduction of the percentage of dead seeds favored by the spectrum BR-LEDs and W-LEDs. The root fresh mass is increased by all lights, except R-LEDs. The fresh mass of the aerial part presents positive results in the FL lamps. Shoot length is favored by the R-LEDs.

1. Introduction

Capsicum peppers are closely related to the Brazilian richness culture and are a valuable part of the biodiversity heritage, being cultivated an immense variety, sizes, colors, flavors and pungences (Neitzke *et al.*, 2008). The Brazilian production is around 11,071 tons (Conab, 2015), being the state of São Paulo considered the largest producer.

Among the factors that regulate plant production, the light plays an important role because it is an important regulator of growth and development of the plant, as it regulates morphological characteristics and acts as an energy source in the primary metabolism and in the photosynthetic process (Simlat *et al.*, 2016). The qualitative or quantitative characteristics of growth and morphogenesis are influenced by the quality of the supplied light, affecting the plants development, mainly, by photomorphogenic alterations (Heo *et al.*, 2002; Rezende *et al.*, 2008).

In recent years, light-emitting diodes (LED) have been widely used as alternative light source potential for plants (Simlat *et al.*, 2016). Among the advantages of LEDs systems are visible light emission and low heat production for long periods, with a specific wavelength, color and lighting flexibility, reduction of electrical consumption and toxic substances, as well as improved lifetime (Carvalho, 2007; Yeh and Chung, 2009). This technology becomes promising for the growth of plants in a controlled environment, such as in tissue culture and also in the supplementation of growth chambers and greenhouses (Yeh and Chung, 2009).

Different wavelengths of light can trigger a variety of responses in plants (Simlat *et al.*, 2016). For example, red, blue, green and white LEDs lights were tested in different species, mainly forest ones, demonstrating the promotion of seed germination and subsequent development (Gonçalves *et al.*, 2006; Victório and Lage, 2009). Red light may promote seed germination and root development (Daud *et al.*, 2013), shoot elongation (Kim *et al.*, 2004; Araújo *et al.*, 2009), fresh mass increment (Sorgato *et al.*, 2016), and an increase in shoot length (Cybularz-Urban *et al.*, 2007), among others. Combinations of blue and red LEDs may promote biomass increase (Gu *et al.*, 2012; Maluta *et al.*, 2013; Da Silva *et al.*, 2016) and increase of the root system (Gu *et al.*, 2012). The blue wavelengths tend to improve stomatal conductance (Hogewoning *et al.*, 2010), affect phototropism (Johkan *et al.*, 2010), and increase the rate of photosynthetic pigments production. However, plants exhibit a wide range of morphological and phytochemical plasticity in response to each type of wavelength of light (Macedo *et al.*, 2011).

Many studies report that LEDs can modify seed germination and plant growth and development (Gonçalves *et al.*, 2006; Victório and Lage, 2009; Daud *et al.*, 2013; Da Silva *et al.*, 2016). Although there are reports on the development of seedlings of the genus *Capsicum spp.* in light qualities (Da Silva *et al.*, 2016), the effects of the light spectral qualities on the germination and vigor of *Capsicum chinense* have not yet been analyzed. Thus, the objective of this work was to verify the impact of the light spectral qualities on the physiological quality of *Capsicum chinense* seeds.

2. Materials and Methods

Plant material and conduction of the experiment

The work was conducted at the Plant Tissue Culture Laboratory of the Universidade Federal de

Santa Maria, campus Frederico Westphalen - RS (Federal University of Santa Maria, campus Frederico Westphalen - RS), in november 2016.

The experiment was conducted in a completely randomized design, in a 4x5 factorial scheme, with four pepper cultivars (*Capsicum chinense*) and five light spectral qualities, totaling 20 treatments, with four replicates of 50 seeds each treatment, totalizing 4000 seeds tested. Four cultivars of pepper were used [BRS Moema biquinho yellow (Biq. Yellow), Airetama biquinho red (Biq. Red), Boyra Habanero red (Boyra Hab. Red) and BRS Seriema tupã bode red (Tupã Bode Red)], and five light spectral qualities [TEC-LAMP® blue LEDs - (450 nm) B-LEDs; red LEDs (660 nm) R-LEDs; blue (450 nm) + red (660 nm) BR-LEDs in the ratio of 40% and 60%, respectively; white LEDs W-LEDs; and special daylight type fluorescent FL (Osram®, Brazil)].

The seeds were placed inside gerbox® boxes with lids (11x11x3 cm) containing two sheets of Germitest® paper (in box dimensions), moistened with 0.2% KNO₃ solution (dissolved in distilled water), in proportion to 2.5 times the dry paper weight, as described in the Regras Análise de Sementes (Rules for Seed Analysis) (MAPA, 2009). The gerbox boxes were maintained in a growth room under temperature of 25±2°C and a luminous intensity of 36 µmol m⁻² s⁻¹ for 14 days.

Analyzed variables

For the germination test, counting was performed by seven and 14 days after the test installation. At the first count (FC), the normal seedlings were counted and the values expressed as a percentage (%), at 14 days the following variables were analyzed: percentage of germination (PG), percentage of abnormal seedlings (PAS), percentage of hard seed (PHS) and percentage of dead seeds (PDS) (MAPA, 2009). According to MAPA (2009), dead seeds are the seeds that do not germinate at the end of the test, are neither hard nor dormant, and are usually softened, attacked by microorganisms and show no signs of germination. Already, the hard seeds are those that remain without absorbing water for a longer period than normal and are therefore at the end of the test with the appearance of seeds newly placed on the substrate.

For the root length (RL) and shoot length (SL) variables, 10 seedlings of each replicate were measured for all light qualities, being measured with a digital caliper. For the fresh mass of the aerial part (FMAP) and root fresh mass (RFM), the same seedlings were

used for the SL and RL measurement, with the values referring to the 10 seedlings. Afterwards, the seedlings were conditioned in paper bags and kept in a forced air oven at 60°C, until constant weight was reached, to determine the dry mass of shoot (DMS) and dry mass of the root (DMR).

The germination speed index (GSI) was calculated by the sum of the number of germinated seeds per day, divided by the number of days between sowing and germination, following the Maguire's methodology (1962).

$$GSI = (G1 / N1) + (G2 / N2) + \dots + (Gn / Nn) \quad (1)$$

Where GSI = G_1, G_2, \dots, G_n = number of seedlings computed in the first, second, third and last count; N_1, N_2, \dots, N_n = number of days of sowing to the first, second, third and last count.

The obtained data were submitted to analysis of variance, and the interaction between pepper cultivars and light spectral qualities was evaluated, and when they were significant, the averages were compared by the Tukey's test, at 5% of error probability, using the statistical program Assistat 7.7 beta.

3. Results

The analysis of variance showed significant interaction for the pepper cultivar factors x light spectral qualities only for the fresh mass of the aerial part (FMAP) and shoot length (SL) variables. The variables of the first count (FC), percentage of germination (PG), normal seeds (NS), abnormal seeds (AS), hard seeds (PHS), germination speed index (GSI), root fresh mass (RFM) and root length (RL) were significant only for the pepper cultivars factor. Root length (RL), root fresh mass and percentage of dead seeds (PDS) variables were significant for the light spectral qualities factor. On the other hand, the dry mass of the aerial part (DMAP) and dry mass of the root (DMR) variables were not significant (data not shown) by the F test, at 5% of error probability.

The pepper cultivars differed for the percentage of normal seeds (NS) and abnormal seeds (AS). The highest percentages of NS were observed for Biq. Yellow pepper with approximately 88%, being the same as Biq. Red pepper (85%), and differing from the others ($p < 0.05$) (Fig. 1A). For the variable AS, the Tupã Bode Red cultivar presented the highest values, with 12.30% of abnormality, being higher than the others, and the lowest percentages were verified for Biq. Yellow pepper, with 4.40% (Fig. 1B).

Regarding the percentage of hard seeds, the Boyra

Hab. Red cultivar had the highest values, with an average of 11.8%, differing significantly from the others (Fig. 1C). The Biq. Yellow pepper cultivar showed the highest percentages of germination in the evaluation of the first count (FC), with 94.8%, differing significantly from the Boyra Hab. Red cultivar (Fig. 1D).

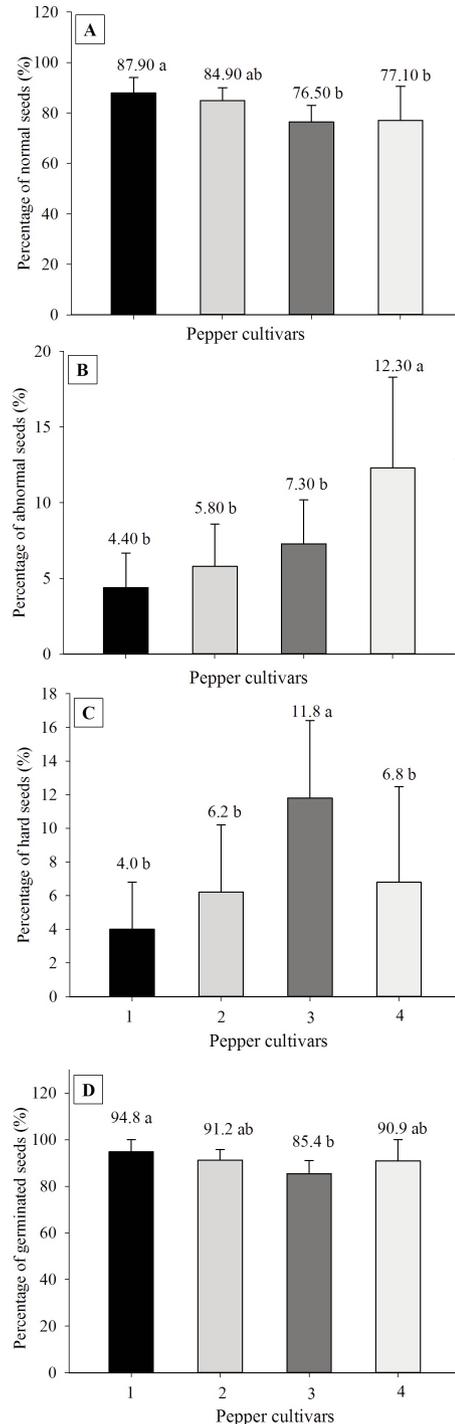


Fig. 1 - Percentage of normal seeds (NS-A), abnormal seeds (AS-B), hard seeds (HS-C) and germinated (GS-D) of four pepper cultivars, being Biq. Yellow, Biq. Red, Boyra Hab. Red and Tupã Bode Red, submitted to different light spectral qualities. *Different letters represent significant statistical difference (Tukey's Test $P < 0.05$; Bars=SD).

For the root length variable, the Boyra Hab. pepper presented the highest values with approximately 51 mm in length, being statistically different from the other cultivars. The lowest averages were observed in Biq. Yellow and Biq. Red peppers with 36 and 35 mm, respectively (Fig. 2A). The Boyra Hab. Red pep-

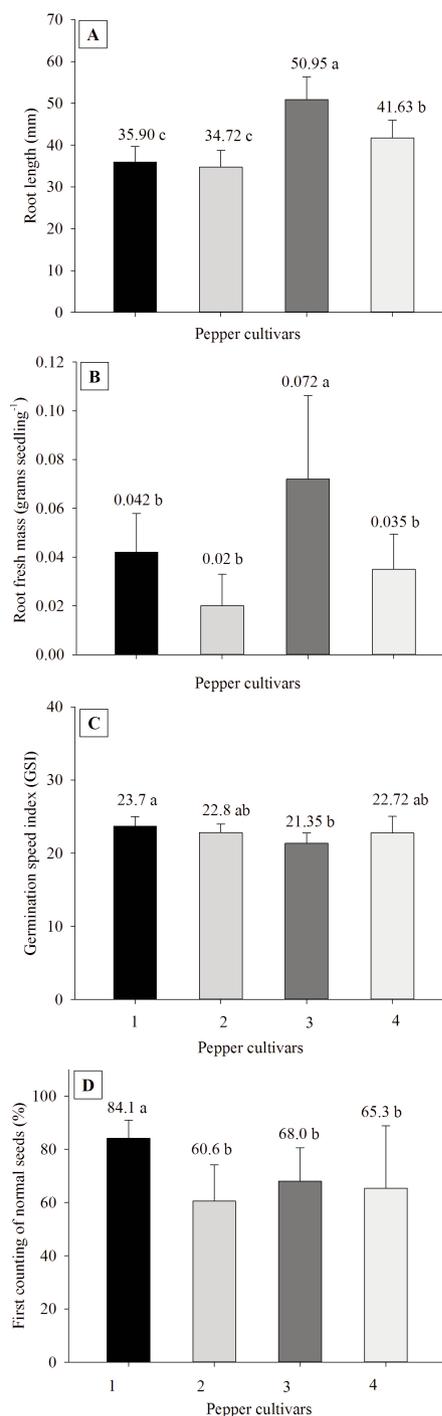


Fig. 2 - Root length (A), root fresh mass (g for 10 seedlings) (B), germination speed index (C) and first counting of normal seeds (D) of four pepper cultivars, being Biq. Yellow, Biq. Red, Boyra Hab. Red and Tupã Bode Red, submitted to different light spectral qualities. *Different letters represent significant statistical difference (Tukey's Test $P < 0.05$; Bars=SD).

per again stood out for the root fresh mass variable, with 0.072 gram for 10 seedling differing significantly from the other ones evaluated (Fig. 2B).

For the germination speed index (GSI), the Biq. Yellow pepper stood out, presenting 23.70, being significantly similar to the Biq. Red and Tupã Bode Red peppers, differing only from Boyra Hab. Red pepper (Fig. 2C). For the first counting of normal seeds the Biq. Yellow pepper showed the highest values with 84.1% of normal seedlings, differing from the other ones (Fig. 2D).

The light spectral qualities of BR-LEDs and W-LEDs provided higher root length (RL), with 43.37 and 42.97 mm respectively, differing statistically from the FL (Fig. 3A). For the root fresh mass variable, it was observed that the red spectrum promoted mass

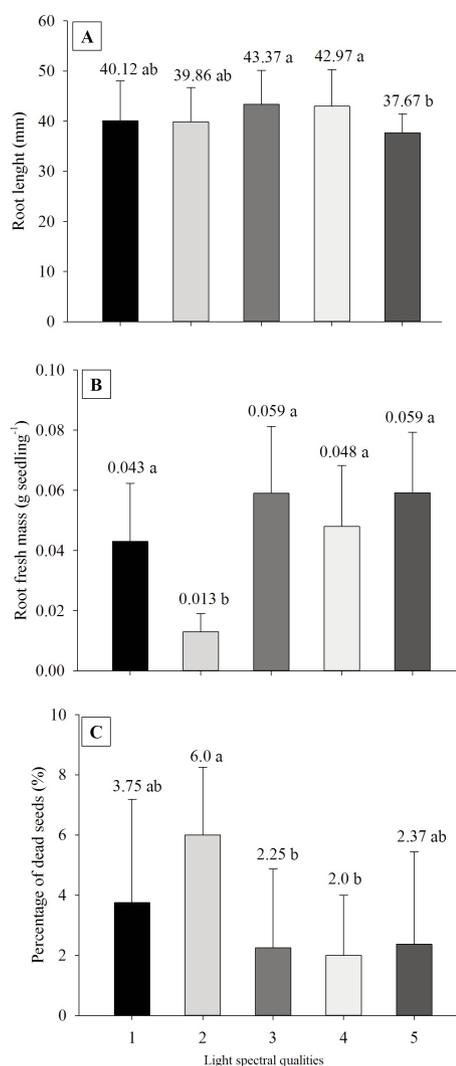


Fig. 3 - Root length (A), root fresh mass (g for 10 seedlings) (B) and percentage of dead seeds (C) of four pepper cultivars submitted to different light spectral qualities, being B-LEDs, R-LEDs, BR-LEDs, W-LEDs and FL lamps. *Different letters represent significant statistical difference (Tukey's Test $P < 0.05$; Bars=SD).

reduction, with 0.013 gram for 10 seedling, differing statistically from the other light spectra, which presented higher mass (Fig. 3B). Correspondingly, it was observed that the red spectrum conditioned the highest percentage of dead seeds, with 6%, differing statistically from the BR-LEDs and W-LEDs. The BE-LEDs and W-LEDs spectra conditioned low percentage of seed mortality (Fig. 3C).

For the Biq. Yellow pepper cultivar the light spectral qualities of W-LEDs, FL and BR-LEDs showed the highest values of fresh mass of the aerial part (FMAP), differing significantly from R-LEDs (Table 1). For Biq. Red and Boyra Hab. Red peppers, the FL light provided greater accumulation of fresh mass, presenting 0.194 and 0.283 gram for 10 seedlings, respectively, being statistically higher to the others (Table 1). As for the Tupã Bode Red pepper, the W-LEDs light conditioned the largest fresh mass of the aerial part, differing significantly from the B-LEDs and R-LEDs (Table 1).

It was observed that the BR-LEDs and FL lights provided a greater increment of fresh mass for Boyra Hab. Red pepper, with 0.236 and 0.283 g for 10 seedling, respectively, differing significantly from the R-LEDs spectrum. The tested peppers presented similar responses in relation to the W-LEDs light spectrum, not statistically different from each other

(Table 1).

R-LEDs spectral quality provided the highest averages for the shoot length variable, being higher to the other spectra. In this spectrum, the Boyra Hab. Red and Tupã Bode Red were superior to the other peppers. For Biq. Yellow pepper the R-LEDs, FL lamp and BR-LEDs spectra conditioned the larger shoot length, statistically differing from the B-LEDs and W-LEDs. For Biq. red pepper, the R-LEDs spectrum was statistically superior to the others (Table 2).

The Boyra Hab. Red pepper presented superior performance to the others, presenting the highest average in shoot length (SL), being favored by the LEDs spectra and in disadvantage by the FL, differing significantly. Shoot length of Tupã Bode Red cultivar was favored by the R-LEDs light spectra qualities, differing significantly from the other spectra (Table 2).

4. Discussion and Conclusions

The Biq. Yellow pepper cultivar was superior to GSI, FC, NS, PG, presenting the lowest percentage of abnormal (AS) and hard (HS) seeds (Fig. 1). However, the Boyra Hab. Red pepper cultivar has been highlighted for the RL (Figs. 2A, 3A), SL (Table 2), FMAP (Table 1), RFM (Figs 2B, 3B) variables. The results sug-

Table 1 - Fresh mass of the aerial part (g for 10 seedlings) of four pepper cultivars, being Biquinho Yellow, Biq. Red, Boyra Hab. Red and Tupã Bode Red, submitted to different light spectral qualities, B-LEDs, R-LEDs, BR-LEDs, W-LEDs and FL lamps

Cultivars	Fresh mass of the areal parts				
	B-LEDs	R-LEDs	BR-LEDs	W-LEDs	FL
Biq. Yellow	0.144 bAB	0.114 bB	0.182 bA	0.191 aA	0.192 bA
Biq. Red	0.1233 bC	0.139 abBC	0.179 bAB	0.169 aABC	0.194 bA
Boyra Hab. Red	0.196 aBC	0.176 aC	0.236 aAB	0.204 aBC	0.283 aA
Tupã Bode Red	0.099 bC	0.138 abBC	0.160 bAB	0.200 aA	0.183 bAB
CV	15%				

* Different lowercase letters in the column or uppercase letters in the row represent significant statistical difference (Tukey's Test $P < 0.05$).

Table 2 - Shoot length of four pepper cultivars, being Biquinho Yellow, Biq. Red, Boyra Hab. Red and Tupã Bode Red, submitted to different light spectral qualities, being B-LEDs, R-LEDs, BR-LEDs, W-LEDs and FL lamps

Cultivars	Shoot length (mm)				
	B-LEDs	R-LEDs	BR-LEDs	W-LEDs	FL
Biq. Yellow	28.66 abB	34.28 bA	30.62 abAB	29.26 bcB	34.15 aA
Biq. Red	26.56 bC	37.65 bA	30.34 bBC	30.65 abBC	33.33 aB
Boyra Hab. Red	30.74 aB	43.14 aA	34.59 aB	33.92 aB	25.52 bC
Tupã Bode Red	29.075 abC	42.10 aA	33.89 abB	26.18 cC	26.53 bC
CV	6.71%				

* Different lowercase letters in the column or uppercase letters in the row represent significant statistical difference (Tukey's Test $P < 0.05$).

gest that Biq. Yellow pepper has a higher germinative potential, while Boyra Hab. Red pepper has greater vigor. The characteristics of germination and vigor are individual for each cultivar and variables between them. For most of them, the speed, uniformity and germination rate depends on external and internal factors to the seed (Plue *et al.*, 2010; Demotes-Mainard *et al.*, 2016), such as temperature, humidity, light, viability of the embryo and genetic factors, characterizing in this way, the differences verified between the pepper cultivars. In general, the use of high vigor seeds results in a good performance of the crops in the field through better establishment of seedlings and survival of seedlings. In this way, germination and vigor tests are important in order to choose the best pepper to be used.

Specific light spectra can act positively stimulating the germination process in some species (Gonçalves *et al.*, 2006), or can be indifferent to others. In general, species whose seeds present sensitivity to the light quality, the positive photoblastics, are considered pioneers in nature, since they require light stimulus to initiate their germination process (Rebouças and Santos, 2008). For the *Capsicum chinense* pepper plant, as observed in this study, only the percentage of dead seeds was influenced by the luminous spectra tested where the R-LEDs spectrum caused a high number of dead seeds whilst the BR-LEDs and W-LEDs reduced the percentage of mortality. The other germination variables evaluated did not present responses to the spectra. Red light has been reported to stimulate seed germination and root development (Bewley and Black, 1994; Abdullateef and Osman, 2011; Daud *et al.*, 2013).

In addition to the quality, the luminous intensity in which the seeds and plants are submitted can also promote differentiated responses in the plant, as verified for *Capsicum chinense* Habanero, which presented increase in growth with the light intensity of $28 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Barrales-López *et al.*, 2015). The luminous spectra used in this experiment were larger, with $36 \mu\text{mol m}^{-2} \text{s}^{-1}$, which may have masked the plant response. It is known that light in excess can result in reduction of the net photosynthetic rate, causing oxidative damage to the foliar tissues; only under appropriate light plants can be fully self-regulated to obtain the best status for absorption and transformation of light energy (Yao *et al.*, 2017). It is known that excess light can also promote photo-voltaic changes in plants, leading to the production of reactive oxygen species (ROS), which may have promoted mortality in seeds submitted to the red spec-

trum.

After germination, it was possible to observe changes in the morphological characteristics due to the different light spectral qualities in which they were submitted. Fluorescent light (FL), for example, provided a higher increase of FMAP in the evaluated peppers. This spectral quality is the most used for *in vitro* growth of plant species. Positive results in the increase of fresh mass of the aerial part were already found for *Curcuma longa* in fluorescent light, followed by red light ($\sim 625\text{-}440\text{nm}$) and yellow light ($\sim 565\text{-}590\text{nm}$) (De Souza Ferrari *et al.*, 2016). Naturally plants develop themselves under varied lights composed of a mixture of quality and quantity, which promotes the activation of several photoreceptors, among them phytochromes (Rockwell *et al.*, 2006).

The highest values of shoot length were verified in R-LEDs spectral quality for *Capsicum chinense* peppers, corroborating with other studies, which found an increase in root formation in cultures such as *Jatropha curcas* and *Protea cynaroides* (Daud *et al.*, 2013; Wu and Lin, 2013), and *Stevia rebaudiana* (Simlat *et al.*, 2016). Kim *et al.* (2004), observed stretching of the aerial part of chrysanthemums cultivated *in vitro*, under light in the red band. When the plants were submitted to the R-LEDs spectrum, some authors verified elongation in *Cattleya loddigesii* (Araújo *et al.*, 2009), increase of fresh mass in *Dendrobium phalaenopsis* (Sorgato *et al.*, 2016) and increase of shoot length for *Cattleya* (Cybularz - Urban *et al.*, 2007), corroborating with the results found in this work.

Red light is effective for photosynthesis as the red emission spectrum fits perfectly with the photon energy required to reach the first excited status of *a* and *b* chlorophyll (Singh *et al.*, 2015). Lights in blue and red spectra too are strongly absorbed by phytochrome through specific photoreceptors (Mathews, 2010). These photoreceptors activate enzymes associated with the synthesis of auxins, growth hormone, and greater photosynthetic efficiency (Sun *et al.*, 1998), promoting an increase in growth, justifying the results found. In this way, plants that grow under these conditions have a good initial development, such as a well-formed root system, which allows for faster acclimation and better survival rates in the field (Chandra *et al.*, 2010; Gruszecki *et al.*, 2010).

Positive results for root fresh mass and root length were observed in BR-LEDs spectrum. Some studies indicate that combinations of LEDs with blue (30%) and red (70%) spectrum promoted an increase

in biomass of *Fragaria x ananassa* and *Saccharum officinarum* (Nhut et al., 2003; Maluta et al., 2013). Results for *Anthurium andraeanum* favored gains in fresh and dry matter in the combination of RB-LEDs, followed by white light (Gu et al., 2012). When RB-LEDs have been used *in vitro* propagation, a large part of the works observed a mass gain in both the root and the aerial part (Gu et al., 2012; Maluta et al., 2013; Da Silva et al., 2016).

For chili culture (*Capsicum annuum* L. cv. Rubi Gigante), shoot length and collar diameter were favored by treatment with BR-LEDs light compared to white fluorescent light (FL) (Da Silva et al., 2016). For this species, the authors also point out that the different qualities of the light spectrum have little effect on the growth and development of seedlings.

The photosynthetic pigments absorb light in the red and blue range, the red quality increases the photosynthetic rate, while blue quality improves the chloroplast development, chlorophyll biosynthesis and stomatal opening, thus, increasing the content of photosynthetic pigments (Johkan et al., 2010; Hogewoning et al., 2010; Daud et al., 2013). These luminous spectra influence the primary and secondary metabolism in plant development, however, plants exhibit a wide range of morphological plasticity, and phytochemical in response to a given type and wavelength of light (Macedo et al., 2011). In this way, some cultures are favored by the supply of these wavelengths, increasing their growth.

The tested peppers showed good results for the root length and root fresh mass variables when submitted to the W-LEDs spectrum. Positive results with the use of W-LEDs were obtained by Wilken et al. (2014), in which they found more vigorous growth of *Musa spp.* compared to the use of fluorescent lamps. For sugarcane plants, W-LEDs lamps promoted a higher number of shoots, in addition to a higher content of chlorophylls and carotenoids (Ferreira et al., 2017).

The emitted photons in the combination of BR-LEDs, suppose that more photoreceptors of the pepper seedlings received stimuli, which may have triggered some morphogenetic mechanism in more photoreceptor cells than when exposed to only one spectrum. Similar assumptions were made by Shimokawa et al. (2014) and Chen et al. (2017). In this way, the positive results found for RFM and RL and the low seed mortality (SM), in the BR-LEDs combination were explained.

Light is a signal that is received by photoreceptors, which regulate plant differentiation and growth

(Li et al., 2013). The quality of the emitted light by LEDs has promoted significant improvements in morphogenesis and differentiation in different species grown *in vitro* (Gupta and Jatothu, 2013). However, the effects and mechanisms associated with light quality may be peculiar to each species or cultivar (Li et al., 2013; Da Silva et al., 2016). Some explanations may be generalized, however, this specificity seems to be related to the different responses that were found for the vigor of the pepper cultivars.

The Big. Yellow and Boyra Hab. Red present high potential of germination and vigor, respectively, indicating high physiological quality.

In general, the light spectral qualities provide differentiated responses in the initial development of the peppers, being the reduction of the percentage of dead seeds favored by the spectrum BR-LEDs and W-LEDs. The root fresh mass is increased by the all lights, except R-LEDs. The fresh mass of the aerial part presents positive results in the FL lamps. Shoot length is favored by the R-LEDs.

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