



Physiological characteristics of endive, lettuce and chicory seeds as a function of spectral quality

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Abstract

Seedling production is a critical step in the establishment of vegetables. This work aimed to evaluate the effect of different spectral qualities on the germination and vigor of endive, lettuce, and chicory seeds. The experiment was carried out in three stages. The first stage, two cultivars of lettuce ('Crespa Repolhuda' and 'Vera') and six spectral qualities (blue LED, red LED, blue + red LED, white LED, fluorescent and dark) were evaluated; the second stage, two cultivars of chicory ('Lisa Escarola' and 'Palla Rosa') and the same spectral qualities were evaluated. In the third stage, the spectral quality of the endive cultivar 'Pão de Açúcar' was evaluated. The experiments were performed in a completely randomized design, with four replications of 50 seeds each. The evaluated parameters were: germination percentage, first count, germination speed index, seedling length, and fresh and dry mass. Endive, lettuce and chicory seeds germinated both in the presence or not of light so that they can be classified as neutral photos. A spectral LED of red quality fostered the development of the most significant volume of fresh mass on the endive, lettuce, and chicory. All the spectral qualities stimulated root growth. The dark, on the other hand, promoted the most significant length of the aerial part, promoting seedlings etiolation.

Keywords: *Cichorium endivia* L., *Cichorium intybus* L., *Lactuca sativa*, seeds, germination

Introduction

Chicory and almond stand out, as with the most popular leafy vegetables and most used as salads. Their crops are concentrated close to large cities, which facilitates the direct sale of producers to retail and food services. Besides, chicory crops require a low initial investment and provide a short-term return, which are core strategic factors for agribusiness (Costa et al., 2007; Trani et al., 2014).

Leafy vegetables propagate sexually. For this reason, their germination is crucial for the production of seedlings, a critical stage for the establishment of crops (Melo & Varela, 2006; Boo et al., 2011).

Several environmental factors, including luminosity, influence germination. The quality of the light received by the seeds acts as a biological mechanism that varies according to the species, promoting or inhibiting germination (Pons, 1991). The germination

luminosity requirement is an element to classify seeds. The seeds can be positive photoblastic when they require light to germinate, negative photoblastic when germination occurs in the absence of light, and neutrals that do not depend on luminosity to germinate (Sousa et al., 2008; Meiado, 2012).

The perception of light occurs through the phytochrome molecule, a protein that has the function of a signal receiver that captures light signals that may or may not trigger seed germination (Souza, 2008). The differential absorption of light spectrum waves by the seeds regulates germination: the active red light allows germination, as long as the distant red light inhibits germination (Taiz et al., 2017).

The use of light sources with different wavelengths from light-emitting diodes (LEDs) for plants has attracted considerable interest in recent years, mainly focused on vegetables, as improving the initial establishment and,

consequently, the final quality of the plants, in addition to their high potential for commercial application (Lin et al., 2013; Koksai et al., 2015).

Blue and red LEDs positively influenced the production of cucumber seedlings (Silva et al., 2016). The dry mass of saffron seedlings increased, as submitted to white and yellow spectra (Ferrari et al., 2016). However, the absence of light promoted a higher rate of germination speed in janaguba seeds (Amaro et al., 2006). *Euphorbiacea* spp. seeds (*E. heterophylla*, *E. hysopifolia*, and *E. hirta*) need light to trigger the germination process (Ferreira et al., 2017).

The literature describes few reports on the use of LED lights in seeds and the growing use of this technology in the production of seedlings. This work aimed to evaluate the effect of different spectral qualities in the germination and vigor of almond, lettuce, and chicory seeds.

Material and Methods

Plant material and experiment conditions

The work was carried out in a growth room at the Federal University of Santa Maria, Frederico Westphalen campus - RS, during September 2017, with a randomized, open experimental design, with four replications of 50 seeds. Three tests were carried out with leafy vegetables of the Asteraceae Family: lettuce, chicory, and endive.

The first test was performed in a 2x6 factorial scheme, where the cultivars of lettuce Vera and Crespa Repolhuda were submitted to six light qualities: TEC-LAMP® LED blue (450 nm); Red LED (660 nm); Blue LED (450 nm - 40%) + red (660nm - 60%); White LED; special daylight fluorescent lamp (Osram®, Brazil); and absence of light (Dark). The dormancy break was carried out under fluorescent light for two hours (before starting the tests), the paper being moistened with a solution of 0.2% KNO₃ (dissolved in distilled water), in the proportion of 2.5 times the weight of the dry paper, as described in the Rules for Seed Analysis (RAS) (Brazil, 2009) for breaking dormancy.

In the second test, almond seeds, cultivar Pão de Açúcar, were evaluated in the same light qualities tested for lettuce. The third test was conducted in a 2x6 factorial scheme, where the chicory cultivars Lisa Escarola and Palla Rosa were evaluated, in the same six-light qualities tested for lettuce and endive.

In the three tests performed, the seeds were placed on two sheets of Germitest® paper inside gerbox® boxes with lids (11x11x3cm). The gerbox® boxes were placed in a growth room at a temperature of 25 ± 2 °C and light intensity of 36 μmol m⁻² s⁻¹, for seven days for lettuce cultivars and 14 days for chicory and almond cultivars, as described in the RAS protocol (Brazil, 2009).

Variables analyzed

For the germination test, the first count of the lettuce was performed four days after the installation of the experiment and the last count at seven days. For almond and chicory, the first count was performed at five days and the last at 14 days. The first count also assessed the percentage of healthy seedlings.

The destructive analysis was carried out on the last evaluation day, analyzing the following variables: percentage of germination, percentage of hard seeds, percentage of abnormal seedlings, and percentage of dead seeds. The germination speed index was calculated by adding the number of seeds germinated for each day, dividing by the number of days between sowing and germination, according to Maguire (1962).

$$\text{GSI} = \left(\frac{G1}{N1}\right) + \left(\frac{G2}{N2}\right) + \dots + \left(\frac{Gn}{Nn}\right)$$

Where: GSI = G1, G2, ..., Gn = number of seedlings computed in the first, second, third, and last count; N1, N2, ..., Nn = number of days from sowing to the first, second, third and last count.

Shoot length and root length were measured on ten seedlings of each repetition by a digital caliper. Fresh seedling mass was assessed on 20 seedlings were used per repetition, to dry mass after they were packed in paper bags and taken to a forced air circulation oven at a temperature of 40 °C, until reaching constant mass, determining the variable dry mass seedling and the results expressed in g 20 seedling⁻¹ (Brazil, 2009).

The Shapiro-Wilk normality test was applied to the data of three experiments separately. After the confirmation of the normal distribution of the data, the analysis of variance (ANOVA) analyzed the interaction between the hardwood cultivars and the spectral qualities. As the interaction displayed significance, the means were compared by the Scott-Knott test at 5% probability of error. All the statistical analysis was performed by the statistical program SISVAR version 5.6 (Ferreira, 2014).

Results

Lettuce

The germination evaluation pointed out superior results in the first and last count of the 'Crespa Repolhuda' variety. The same cultivar displayed the lowest average for the first count as lighted using the white LED. The average final germination values were similar, close to or equal to 100% in all the treatments (Figures 1A and 1B).

In the cultivar Vera, the blue LED induced the lowest average at the first count. As to the final germination, the blue and blue + red LEDs induced the

highest germination percentages (Figures 1A and 1B). The germination speed index was higher in the cultivar *Crespa Repolhuda*, with an average of around 25 in all

spectral qualities. The blue and blue + red LEDs induced the highest results in the cultivar *Vera* (Figure 1C).

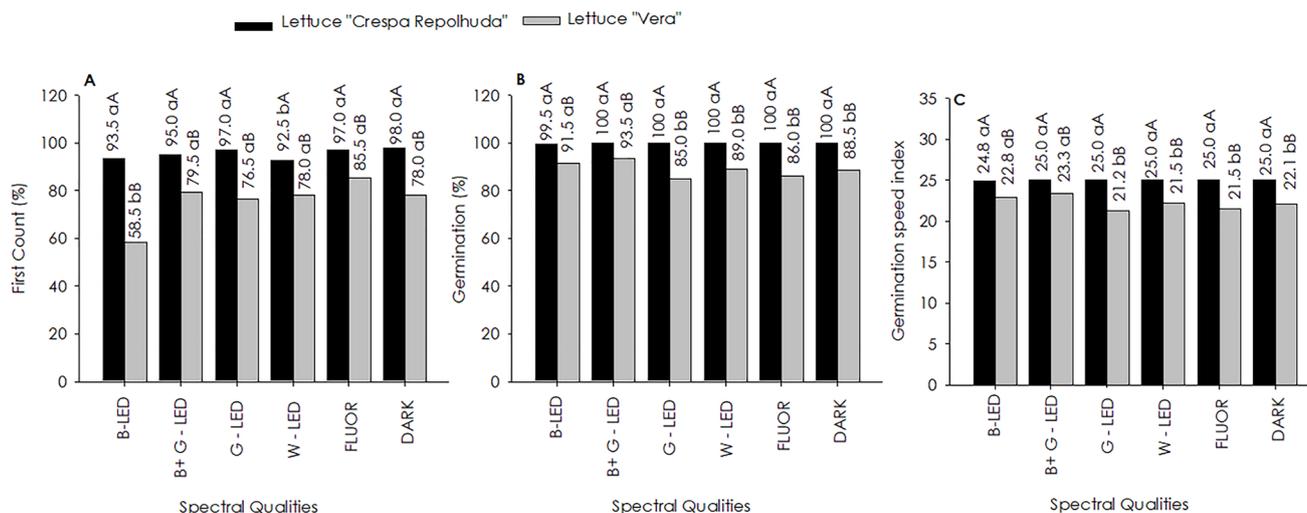


Figure 1. First count (A), germination (B) and germination speed index (C), of two lettuce cultivars submitted to different spectral qualities.

The spectra qualities did not influence the variables percentages of the normal and abnormal seedlings for the cultivar '*Crespa Repolhuda*'. These variables always displayed higher values than the '*Vera*' cultivar. As for the cultivar '*Vera*', the blue LED induced a significant reduction in the percentage of normal seedlings and an increase in the percentage of abnormal seedlings (Figure 2A and 2B).

As the cultivar '*Vera*' was submitted to blue LED light, some of the seeds died. In the other treatments, the variable "dead seeds" was equal to zero (Figure 2C). As for hard seeds, the spectral qualities did not significantly affect the '*Crespa Repolhuda*', quality, while for '*Vera*', there was a higher occurrence of hard seeds in the seeds submitted to the red, white, fluorescent and dark LED lights (Figure 2D).

The shoot length was higher in both cultivars as the seeds were submitted to darkness, and the other qualities were significantly lower. The comparison between the two cultivars highlighted that in the blue LED, the cultivar '*Crespa Repolhuda*' was superior to the cultivar '*Vera*'. The other qualities displayed no significant statistical difference (Figure 3A).

As to the root length, the cultivar '*Crespa Repolhuda*' displayed higher values than the cultivar '*Vera*'. The spectral qualities did not affect this variable (Figure 3B).

The fresh seedling mass did not differ significantly among the treatments for '*Crespa Repolhuda*'. The '*Vera*' variety displayed the highest average values as submitted to red and dark treatments (Figure 3C). For the dry mass of seedlings, the spectral qualities did not

influence neither of the cultivars studied. When comparing both cultivars, in the blue LED and white LED qualities, '*Crespa Repolhuda*' cultivar was inferior to '*Vera*' in the other qualities, there was no statistical difference (Figure 3D).

Endive

The results display that in the first count, the blue, blue + red, red, and dark LED treatments had the highest values when compared to the white and fluorescent LEDs (Table 1). The data highlight that the qualities influence the germination of the seeds, with the white LED inducing a significant reduction in the percentage of germination when compared with the other spectral qualities (Table 1). As to the GSI, the blue LED displayed the lowest average (Table 1).

The variables "normal seedlings", "abnormal seedlings", and "dead seeds" did not differ statistically among the spectral qualities. As to the variable "hard seeds", on the other hand, the blue LED induced a significant reduction in this variable (Table 2).

The spectral qualities affected the length of the almond seedlings. Seedlings grown in the dark developed the most extended aerial parts, followed by those submitted to the red LED and as to the roots, red and blue + red LEDs stimulated the most extended development (Figure 4A). When analyzing the fresh weight variable, we observed that the seedling submitted to red and blue + red LEDs displayed a higher increase in fresh weight compared to the other treatments. As for the dry mass of seedlings, those seedlings grown in the dark displayed the lowest results, in comparison with the other spectral qualities (Figure 4B).

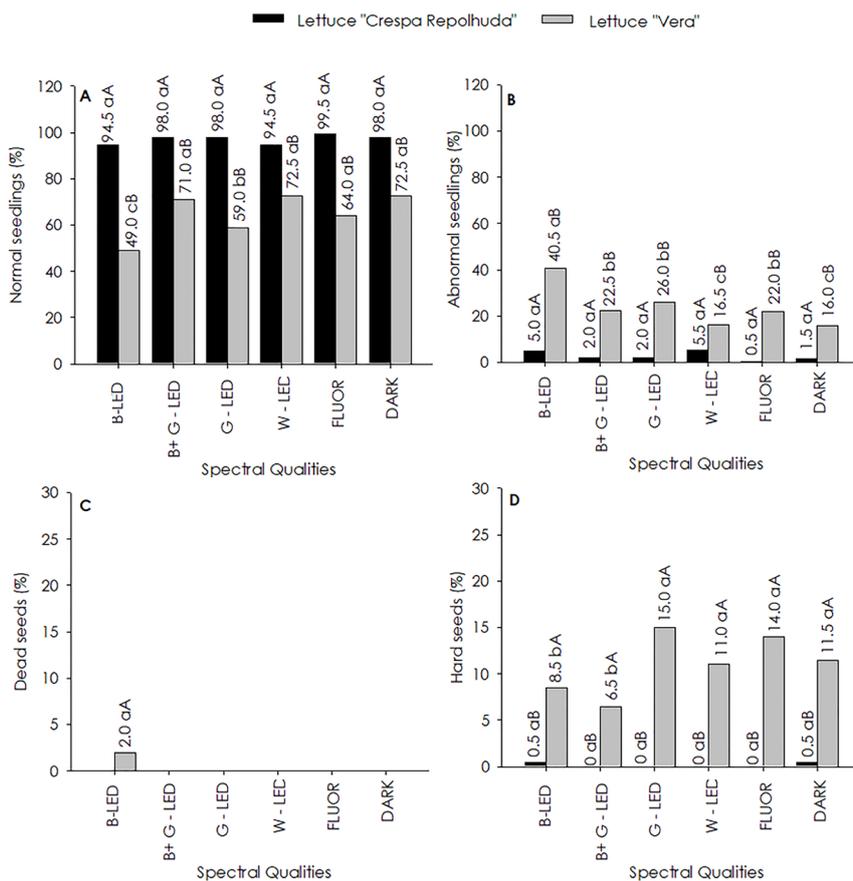


Figure 2. Normal seedlings (A), abnormal seedlings (B), hard seeds (C), and dead seeds (D), from two lettuce cultivars submitted to different spectral qualities.

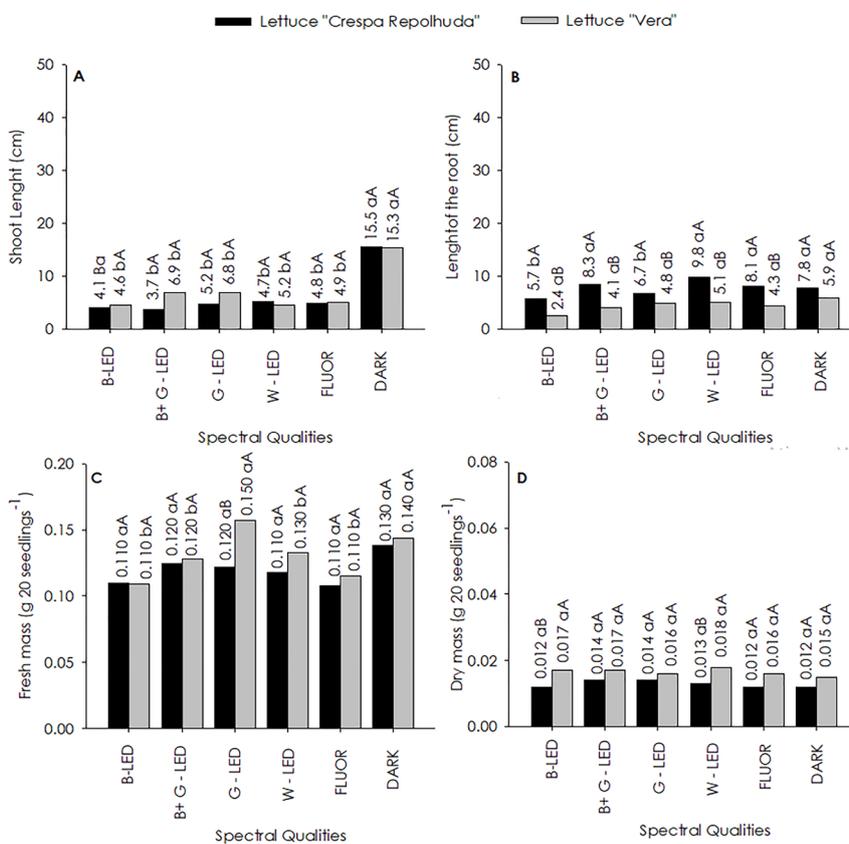


Figure 3. Shoot length (A), root length (B), fresh weight (C), dry weight (D), of two lettuce cultivars submitted to different spectral qualities.

Table 1. First count, germination percentage (A) and germination speed index (B) of the Pão de Açúcar almond when subjected to different spectral qualities.

Spectral qualities	First Count (%)	Germination (%)	Germination speed index
Dark	62.5 a	90.0 a	22.5a
Fluorescent	42.5 b	90.5 a	22.6a
White LED	31.5 b	46.0 b	23.0a
Red LED	66.0 a	91.0 a	22.7a
Blue+red LED	55.5 a	91.0 a	22.7a
Blue LED	69.0 a	851 a	19.6 b

Table 2. Percentage of normal seedlings, abnormal seedlings, dead seeds, and hard seeds of Pão de Açúcar endive submitted to different spectral qualities.

Spectral qualities	Normal seedlings	Abnormal seedling	Dead seeds	Hard seeds
	(%)			
Dark	79.5 a	4.5 a	6.0 a	10.0 a
Fluorescent	76.5 a	8.5 a	5.5 a	9.5 b
White LED	74.0 a	12.0 a	8.0 a	8.0 b
Red LED	79.5 a	5.5 a	6.0 a	9.0 b
Blue+red LED	76.0 a	7.5 a	7.5 a	9.0 b
Blue LED	67.0 a	9.5 a	7.5 a	16.0 a

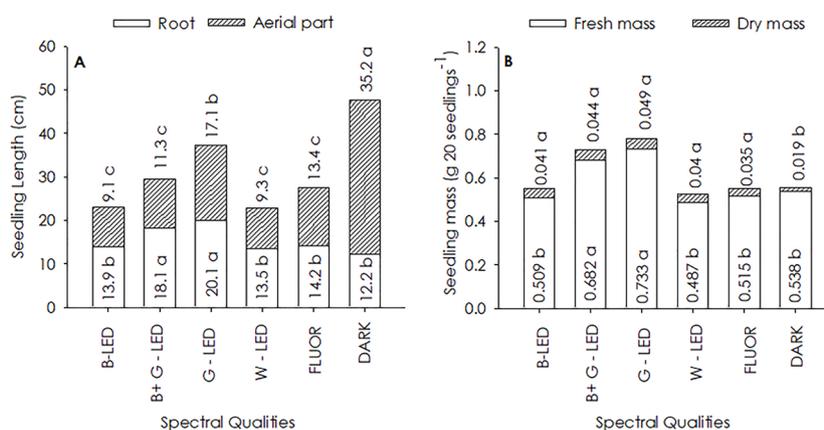


Figure 4. Length of aerial part and root (A) and fresh and dry mass of seedlings (B) of ‘Pão de Açúcar’ almond, submitted to different spectral qualities.

Chicory

Figure 5 shows the results of the first count for the two chicory cultivars. The different types of light did not significantly affect the first count of the cultivar ‘Lisa Escarola’. On the other hand, the cultivar ‘Palla Rosa’ showed a different response, and the dark, blue + red and fluorescent LEDs negatively affected the first count result. When comparing the two cultivars, the difference between the varieties submitted to the treatments dark, fluorescent, and in the blue + red LED differed significantly, with the cultivar ‘Lisa Escarola’ superior to the ‘Palla Rosa’ (Figure 5A).

When evaluating the final germination, it was found that the cultivar Lisa Escarola displayed the lowest results under the blue and red LEDs. ‘Palla Rosa’ did not display a significant difference for the final germination under the different light treatments (Figure 5B).

Spectral qualities influenced the germination speed index. The cultivar ‘Lisa Escarola’ displayed the

lowest averages under the blue and red LEDs. The cultivar ‘Palla Rosa’ displayed the lowest averages as submitted to the dark and red LED treatments (Figure 5C).

The two chicory cultivars showed different responses in all germination variables. In normal seedlings, the spectral qualities did not affect the ‘Palla Rosa’ variety. The performance of this variety was significantly better than the ‘Lisa Escarola’, in all treatments, but fluorescent and dark light, where both cultivars presented a similar percentage. It was in these two treatments the cultivar ‘Lisa Escarola’ displayed average germination above 50%. in the others, the percentage of normal seedlings was below 40% (Figure 6A).

In the cultivar ‘Palla Rosa’, the spectral quality did not affect the percentage of abnormal seedlings, and its performance was higher since the averages found were much lower than the ‘Lisa Escarola’. Besides, the seedlings of this variety submitted to the blue, blue + red, and white LED treatments displayed a higher percentage

of abnormal than normal seedlings (Figure 6B).

The spectral qualities did not influence the percentage of dead seeds for the two cultivars studied. Data displayed a more significant amount of dead seeds in the cultivar Lisa Escarola in the blue LED (Figure 6C).

In the hard seeds, the worst results were found in 'Lisa Escarola', in all spectral qualities, but most notably in the samples submitted to the blue LED and red LED lights. For 'Palla Rosa', there was no influence of spectral qualities on this variable (Figure 6D).

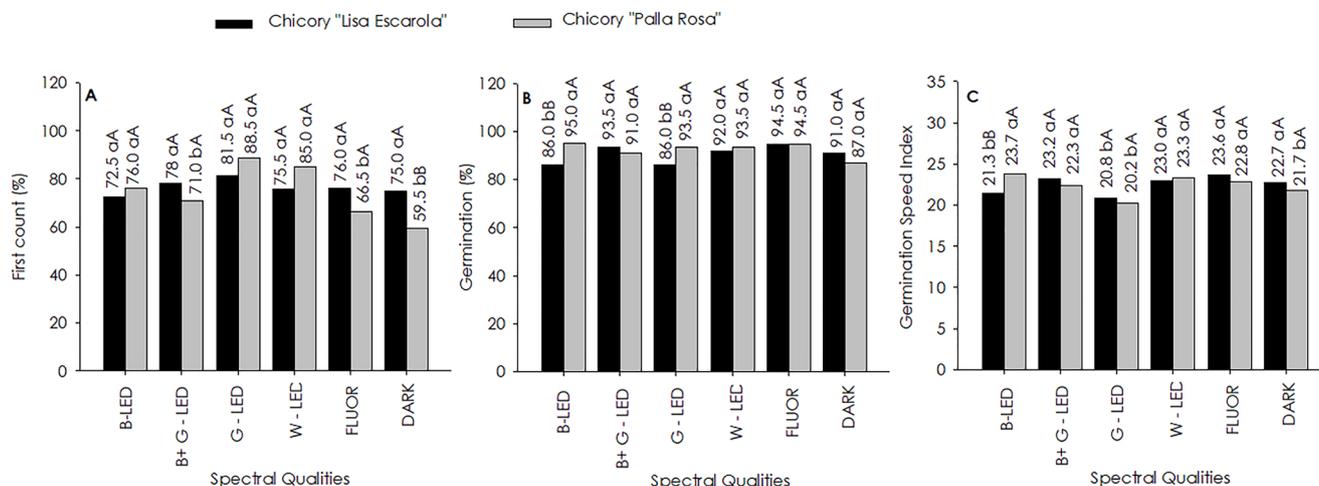


Figure 5. First count (A), germination (B), and germination speed index (C) of two chicory cultivars submitted to different spectral qualities.

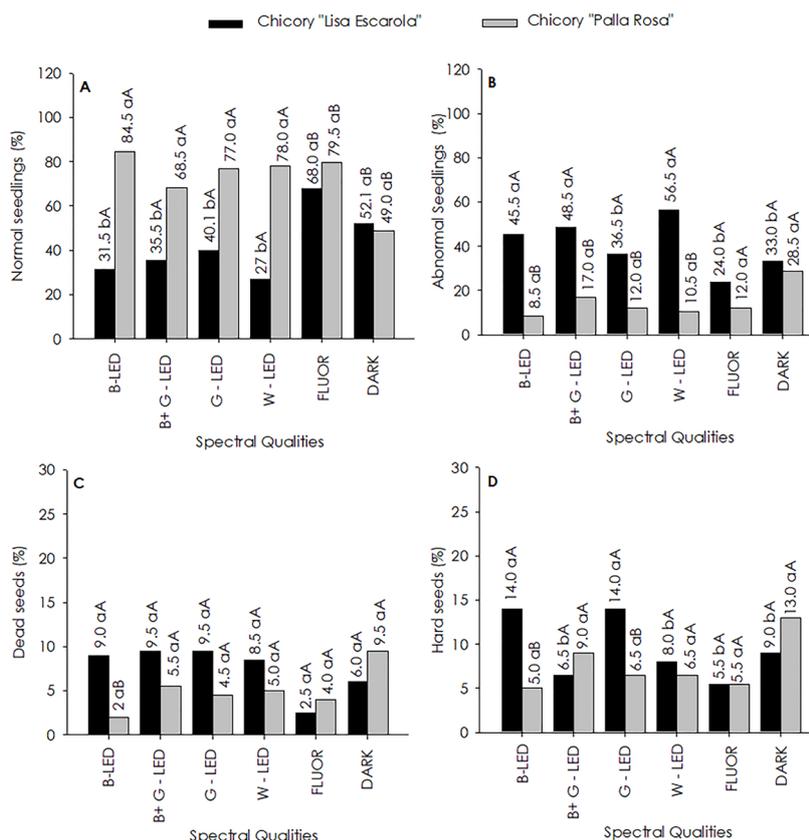


Figure 6. Normal seedlings (A), abnormal seedlings (B), hard seeds (C) and dead seeds (D), of two chicory cultivars submitted to different spectral qualities.

When submitting the chicory cultivars to the different spectral qualities, we observed that the 'Lisa Escarola' had the most extended shoot length in the dark. 'Palla Rosa' also had the highest average in the dark, followed by fluorescent and red LED qualities (Figure 7A).

The root length was always most extensive in

the cultivar 'Lisa Escarola'. In this cultivar, the highest averages were found in the samples submitted to the treatments fluorescent, followed by the red LED. In the cultivar 'Palla Rosa', there was no influence of spectral qualities on the length of the roots (Figure 7B).

The fresh mass of seedlings showed different

performance, and for the cultivar Lisa Escarola the most significant increase was observed in the red LED and the lowest averages in the white LEDs and the dark. As for 'Palla Rosa', the dark, followed by the blue + red LED, displayed the smallest increments (Figure 7C).

The smallest increase in dry mass of chicory seedlings occurred in the dark, for the cultivar Palla Rosa. In contrast, for the 'Lisa Escarola' a similar performance was observed among all spectral qualities (Figure 7D).

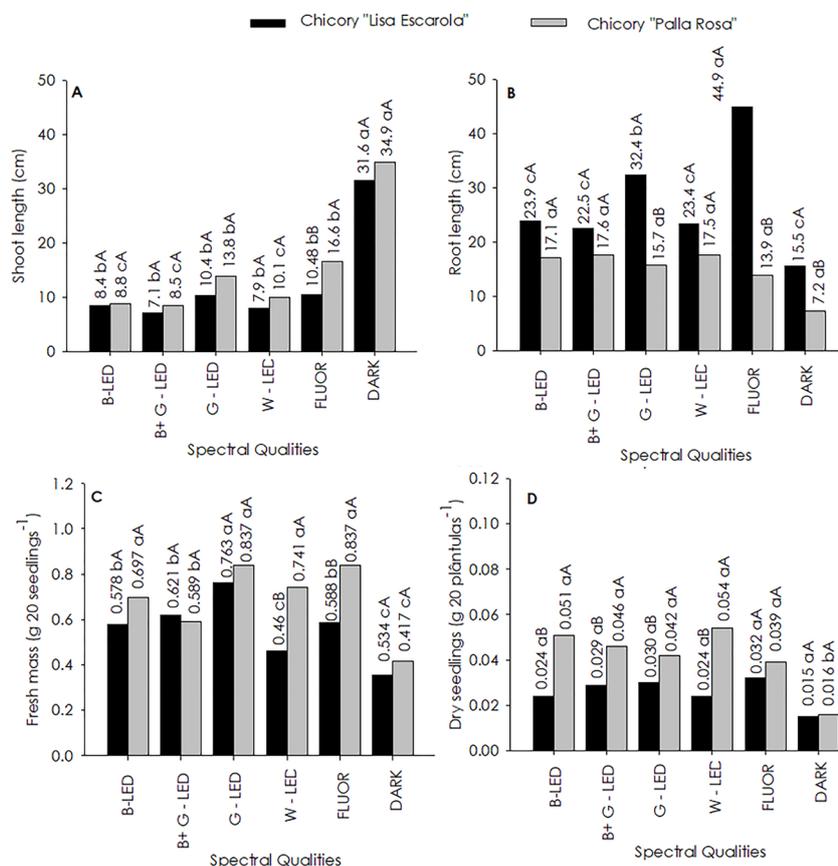


Figure 7. Shoot length (A), root length (B), fresh weight (C), dry weight (D), of two chicory cultivars submitted to different spectral qualities.

Discussion

The germination in the first count indicated that the lettuce, chicory, and almond cultivars showed high averages. Only the lettuce cultivar 'Vera', under the blue LED and in the almond tree under the white LED and fluorescent light displayed low vigor. Santos et al. (2018), when working with the culture of *Chorisia speciosa* found the highest values in the first count in the white and red qualities. The first count variable relates to the number of normal seedlings. It indicates the seed vigor, as it relates to the properties of seeds determining their potential for a rapid and uniform emergence, and the development of normal seedlings under different field conditions (Menezes et al., 2004). According to Tekrony & Egli (1991), in short-cycle crops, like vegetables, the effect of seed vigor on the final productivity and product quality is generally more relevant than in annual crops. In these crops, the harvesting is still carried out at the stage of the vegetative growth of the plant before it enters the reproductive phase.

Light is considered essential for the germination of seeds of various species, indicating its ability to adapt to environmental conditions; thus, plants may have different responses to this environmental factor. In this work, germination was high, reaching values above 85% for all cultivars of lettuce, almond, and chicory, except for the white LED for the almond. In this treatment, the germination was only 46%. As considering the results of the present research, these species of Asteraceae are neutral photoblastics, as the luminosity or its absence does not interfere with germination. According to Castro et al. (2005), some seeds are considered positive or negative photoblastics, however, due to domestication and large-scale commercial production, most commercial cultivars have started acting as neutral photoblastics. However, different results can be found in the literature. Diel et al. (2019) studied the beak pepper, and observed that this species has a high dependence on light to germinate, showing that the species has positive photoblastic features. The phytochrome is the photoreceptor

responsible for absorbing light signals when this factor interferes, as this pigment inhibits or stimulates germination according to the light spectrum received (Yamashita et al., 2011; Galindo et al., 2012).

The germination speed index for the three species that this study analyzes ranged from 19 to 25, with the lowest average being observed in the almond culture, in the blue LED. The chicory 'Palla Rosa', on the other hand, showed the opposite response, displaying the highest germination speed under the blue LED light. According to Silva et al. (2016), neutral photoblastic plants commonly show no significant variations in the germination speed index. According to Nascimento et al. (2011), the germination speed is crucial because as reducing the degree of exposure of seeds and seedlings to the weather. Especially for vegetables, the period between sowing and seedling emergence is critical, and failure in the stand or seedling unevenness can significantly affect the final production and the quality of the vegetable product.

The normal seedlings showed variations among the Asteraceae evaluated. The chicory Escarola in the white LED displayed the lowest average value among the studied species and varieties. In contrast, the 'Crespa Repolhuda' lettuce displayed the highest averages when submitted to the fluorescent light, showing to be associated with the germination process of the cultures. The cultivar of 'Vera' lettuce and chicory escarole showed the highest average percentage of abnormal seedlings. Normal seedlings have all the necessary structures so that they can develop later (Stefanello et al., 2015). These seedlings have a higher capacity for competition with spontaneous plants and survival, even under unfavorable conditions. On the other hand, abnormal seedlings are undesirable in any culture. Abnormal seedling lack essential structures, are defective, they can not compete for the trophic resources, and sensitive to adverse environmental conditions such as soil temperatures and humidity (Nascimento et al., 2011). These seedling are unable to form adult plants with high productive potential (Stefanello et al., 2015).

The variable dead seeds displayed high average values in the chicory culture, these in the qualities blue + red and in the dark. The lettuce cultivar 'Crespa Repolhuda' did not present dead seeds. The seeds are considered dead when they present problems such as embryo deterioration (Ferreira et al., 2006).

The highest percentages of hard seeds were observed in the 'Pão de Açúcar' chicory on the blue LED. No hard seeds were observed in the 'Crespa Repolhuda'

lettuce cultivar in the LED's blue + red, red, white, and fluorescent qualities. Hard seeds are a consequence of integumentary dormancy, impeding the gas exchange that is necessary for germination. Thus the light effect becomes null, as germination was prevented by another factor (Lima et al., 2019).

'Vera' lettuce had a decrease in root growth on the blue LED. This datum is in the counterhand to the description provided by Ferrari et al. (2016) in the saffron culture: the authors described higher value in this quality. The samples submitted to the fluorescent light displayed the highest mean root growth. This observation corroborates with Victorio et al. (2009). The authors found a higher rooting rate for stone breaking culture (*Phyllanthus tenellus*) with this light. Simlat et al. (2016) as studying *Stevia rebaudiana*, and Fontana et al. (2019) in studies with peppers, found that the red spectral quality induced the highest growth of the roots.

The dark treatment induced the highest length of the aerial part in all the studied species: lettuce, chicory, and almond bread cultivars. This datum corroborates with Schmidt et al. (2020), who analyzed the influence of light and dark on the germination of cucurbits, and found that the seedlings Submitted to the dark are stagnant. The fact that the seedlings become etiolate and without the presence of chlorophyll is due to the inhibition of the development of the chloroplasts present in the leaves, which are responsible for photosynthesis and production of photosynthetic pigments (Ramos et al., 2007). The data found disagree from Neves et al. (2018), who described the highest seedling length in *Sapindus saponaria* L. seedlings, submitted to white, red, and distant red light.

As to the fresh mass, lettuce seedlings showed the lowest averages, these in the qualities blue, white LED and fluorescent. The observation corroborates with Ferrari et al. (2016). They, for long turmeric, found the lowest values in the blue LED, but in contrast, the highest value on the white LED, demonstrating that for each species, the result can be divergent under the same spectral quality. The highest average was found in the 'Pão de Açúcar' chicory on the red LED. Most of the vegetable species germinate in the absence of light, but the light is crucial to begin the growth of the seedlings. Therefore, most vegetable seeds should be sown more on the surface of the soil or substrate (Nascimento et al., 2011). According to Lazzarini et al. (2017), the phytochrome absorbs the red spectral quality (in green cotyledons) strongly. It generates a high photosynthetic rate, increasing the production of photoassimilates and increasing the seedlings' mass. According to Mathews (2010), the photoreceptors of the

phytochrome activate the enzymes associated with the synthesis of auxins. This hormone provides growth and an increase in fresh and dry mass, justifying the results found.

The dry mass had the highest increase in white LED and the lowest in the dark, corroborating with Victorio et al. (2009), who, when studying the culture of marigold, also found the most significant increase in the dark. Seedlings, in the absence of light, invest more in stem growth due to high auxinic activity. However, this growth does not result in a real gain in dry mass since the seedlings are just breathing and spending their reserves (Carvalho et al., 2009). This growth compromises the support of the aerial part, justifying the greater fragility of the seedlings germinated in the absence of light.

The quality of the light emitted by the LEDs can trigger numerous physiological responses in seeds, considering that a series of biochemical processes can be activated or turned off by this factor.

The effects and mechanisms associated with the quality of light are peculiar to each species or cultivar and, therefore, studies must be carried out so as not to have losses and negative impacts on the final production of vegetables.

Conclusions

The almond, lettuce and chicory vegetables, from the Asteraceae family, germinated both in the presence and in the absence of light, and can be classified as neutral photoblastic.

The red LED spectral quality led to a higher accumulation of fresh mass in lettuce, almond, and chicory, as well as all spectral qualities stimulated root growth. The dark, on the other hand, promoted too much length of the aerial part, leading the seedlings to etiolation.

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