

The use of photoselective nets affects the leaf characteristics of lettuce cultivars

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Abstract

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The quality of light can influence plant morphogenesis through a series of light receptor mediated processes, principally within the spectral regions of red and blue. Photoselective shading nets combine physical protection with the filtration of specific light wavelengths which can thus alter the light spectrum. The objective of the present study was to analyze changes in density and stomatal morphometry and the concentration of leaf pigments in lettuce cultivars grown in protected environments under different photoselective nets (40% shading). Cultivars of lettuce (Vera, Vanda and Solaris) were grown in a hydroponic system with a laminar flow of nutrients using red, blue and silver nets to provide shading coverage (treatments) in addition to a control with no shading nets. At the end of the production cycle, it was observed that the lettuce cultivars grown under a red net and without a net showed greater stomatal densities and chlorophyll contents. The concentration of photosynthetic pigments (chlorophyll and carotenoid content) showed variations dependent upon the cultivar, demonstrating that the genotypes respond differently to environmental conditions.

Keywords: *Lactuca sativa*; morphometry; stomatal density; photosynthetic pigments

Introduction

For proper development, plants need a degree of cellular plasticity, and the ability to adapt to a wide range of environmental stimuli (Gratani, 2014). Plasticity is one of the main potential factors which can determine the growth and development of plants, especially regarding the assimilation of CO₂ (Pires et al., 2011). Incident solar radiation is a mix-

ture of ultraviolet, photosynthetically active, and infrared radiation, ranging from 280 to 1100 nm (Kiang et al., 2007). Photoautotrophic organisms, such as plants, with the use of photoreceptors categorized as phytochromes can identify various properties of incident light, such as: intensity, quality, direction and duration of exposure (Franklin & Quail, 2010). While phytochromes are predominantly sensitive to red and distant wavelengths, cryptochromes primarily re-

spond to blue and UVA (Yu et al., 2010; Chaves et al., 2011), phototropins to blue light in addition to UVB photoreceptors (Rizzini et al., 2011); plants use information gathered from these receptors to regulate growth and development. These responses are known as photomorphogenesis (Arsovski et al., 2012).

In any natural habitat, the flux density of photosynthetically active photons can vary considerably; plants must develop mechanisms for acclimatization and maintain plasticity in order to adapt to variations of light intensity (Zhang et al., 2003). Alterations in leaf structure constitute critical aspects of a plant's ability of acclimatize and adapt when exposed to different environmental conditions (Schöttler & Tóth, 2014; Ferreira de Melo Júnior & Torres Boeger, 2016). In order to carry out photosynthesis, plants must balance the processes of assimilating carbon dioxide, with water loss via transpiration; the two processes are intrinsically interconnected as they share the same transport path, the stomata (Lee et al., 2016; Pocięcha et al., 2016; Spinelli et al., 2016).

In order to maximize the gas exchange, plants can adjust the number of stomatal pores per epidermal cell during the ontogeny of the leaf in order to regulate the degree of stomatal opening with changes in environment conditions (Raven, 2002). In addition, photosynthetic characteristics generally vary in response to different degrees of irradiance (Schöttler & Tóth, 2014).

The chlorophyll and carotenoid content of leaves is used to estimate the photosynthetic potential of plants via their direct connection to the absorption and transfer of light energy through growth and adaptation to suit different environments. Generally, plants with greater chlorophyll content are able to achieve higher photosynthetic rates due to the reception potential for *quanta* uptake in a given unit of time (Evans, 2013). However, to gain these high photosynthetic rates, we often need to make changes in the intensity and quality of incident light radiation; which may affect the carotenoid content in oleraceous species (Li et al., 2011; Yan et al., 2014).

Nevertheless several authors have confirmed the morphological and physiological effects of light on different plants of commercial interest, the responses vary between species (Macedo et al., 2011; Casierra-Posada et al., 2014; Silva et al., 2015). One way to change the intensity and quality of the radiation that affects the canopy of the plants is through spectral filters; known as photosensitive shading nets which, in addition to physical protection, can filter out specific light wavelengths (Shahak et al., 2004). Given their ability to increase the amount of scattered light, absorb infrared radiation, and alter the light in the spectral regions of the ultraviolet, visible and red-distant, shading nets can be seen to promote morphophysiological changes in plants, which can

result in an increase in vegetative vigor, productivity, quality of branching, variegation of leaves, the overall developmental speed of plants, and the time of flowering (Rajapakse & Shahak, 2007; Stamps, 2009; Arthurs et al., 2013).

Among the photosensitive nets, "colored-ColorNets" (red, yellow, green and blue nets) and so-called "neutral-ColorNets", can absorb relatively shorter or longer spectral bands in the visible range of light (Shahak et al., 2008). The same authors report that these photosensitive nets have been used throughout the world and that their effect on plant cultures has been studied in different climatic regions and under differing methods of cultivation. The effective use of spectral filters depends much upon the knowledge of which spectral bands are involved in the growth responses of a given plant species (Braga et al., 2009).

Taking into consideration that the plants exhibit morphophysiological plasticity in response to environmental changes, primarily those related to different light conditions, the objective of this work was to determine how photosensitive nets influence the morphophysiological parameters of lettuce cultivars.

Materials and Methods

Plant material and experimental conditions

The study was conducted in an experimental area of the Federal University of Santa Maria, *Campus* Frederico Westphalen/ Brazil, with the geographical coordinates: 27° 23' 48" south latitude, 53° 25' 45" west longitude at an altitude of 490m, in the period from June 30 to September 13, 2012. The climate of the region is classified as Cfa, a temperate humid climate with hot summers, according to the classification by Köppen (Alvares et al., 2013). The experiment was carried out in a greenhouse, with the dimensions 10 x 20 m and 3.0 m height and covered with a transparent low-density polyethylene film with 150 µm of thickness and treated for ultraviolet radiation with 87% non-selective transmission.

Seeding was carried out on the 30th of June in phenolic foam, after 24 days, the seedlings were transferred for an intermediate phase until six definitive leaves were produced. In these two steps, the commercial nutrient solution Hidrogood Fert® was prepared and diluted to 50% of its concentration with pH adjusted daily to 6.0. On the 9th of August, 2012, the seedlings were transplanted to a hydroponic system with a laminar flow of nutrients where they remained for 35 days until the point of collection. The nutrient solution was the same as what was used during the initial phases, except maintained at 100% of its concentration, its pH was also adjusted daily to 6.0.

The evaluated cultivars were, Vera, Vanda and Solaris, maintained under four photoselective nets: Chromatinet® blue, Chromatinet® red, Aluminet® silver (all with 40% shading), and without a net (control) (Figure 1). Using these factors, the experiment was conducted in a complete randomized experimental design (DIC) in a factorial arrangement 3 x 4 with a total of 12 treatments, each experimental unit was composed of 44 plants.

Each microenvironment consisted of a production bank composed of 11 hydroponic profiles, each six meters in length, totaling 242 plants and 27 m² of net per microenvironment. The nets were fixed 0.90 m above the hydroponic profiles.

Morphometry and stomatal density

The anatomical aspects of the leaves of lettuce were observed, described and illustrated, with the aid of a camera lucida microscope attachment designed for use with an optical microscope.

The density and morphometry evaluations of stomata were conducted using the last fully expanded leaf in the central region of the leaf blade for all treatments and were collected 35 days after transplantation. Semi-permanent slides of the abaxial and adaxial epidermis of leaves were made by fixing the epidermis with a cyanoacrylate ester on a glass

slide, a technique known as *imprints* (Weyers & Johansen, 1985; Campos et al., 2009).

From these sections, the morphometry and stomatal density were evaluated. For the stomatal density, a technique described by Labouriau et al. (1961) using a camera lucida with a 1 mm² field for counting the stomata of each leaf, 6 samples were examined per treatment with the additional use of an optical microscope Leica model DM 2000.

For the variable morphometry of the stomata [lengths(L, µm), width(W, µm) and area (L*W, µm²)], technique described by Cutter (1987), were observed with a micrometer eyepiece; fifteen samples for each treatment were observed with the aid of an Olympus Microscope Model cx21, and 15 leaves for each treatment were analyzed.

Determination of photosynthetic pigments

In order to determine the levels of chlorophyll *a*, *b*, total and carotenoids, the last fully expanded leaf for six plants of each treatment was removed and analyzed. The thicker veins were removed from the leaves and fragments were separated and weighed in order to obtain three subsamples (0.05 g/leaf) of fresh plant material which was then transferred to assay test tubes which held 5.0 ml of dimethyl sulfoxide (DMSO, 99% purity by volume). The tubes were kept in a water bath



Fig. 1. Cultivars of *Lactuca sativa* (Vera, Vanda and Solaris) kept in (A) greenhouse below (B) different photoselective nets [(C) Chromatinet® blue, (D) Aluminet® silver, (E) Chromatinet® red and (F) without a net (control), in the winter season

with water preheated to 70°C for one hour in order to solubilize the chlorophyll.

After this incubation period, the absorbance (%) of the samples was determined in a spectrophotometer BEL Photonics, model SP acios1105, using 10 mm optical glass cuvettes with a 10 mm optical path (645, 663 and 470 nm), and a blank with only the DMSO solution. The calculations (in mg of chlorophyll per g of fresh foliar plant material) were made according to the following equations, according to Hiscox and Israel stam (1979):

$$\text{Chlorophyll } a = \frac{\{(11,75 * A_{663}) - (2,35 * A_{645})\} * 50}{500} \quad (1)$$

$$\text{Chlorophyll } b = \frac{\{(18,61 * A_{645}) - (3,96 * A_{663})\} * 50}{500} \quad (2)$$

$$\text{Carotenoids} = \frac{\{[(1000 * A_{470}) - (2,27 * Ca) - (81,4 * Cb)]/227\} * 50}{50} \quad (3)$$

$$\text{Total Chlorophyll} = \text{Chlorophyll } a + \text{Chlorophyll } b \quad (4)$$

Statistical analysis

For the evaluations of stomatal density the abaxial and adaxial portions and photosynthetic pigments (chlorophyll *a*, *b*, total and carotenoids), six repetitions per treatments were carried out. For the evaluations of stomatal morphometry (length, width and area), 15 repetitions per treatment were used.

All analyzed variables were submitted to the test of Shapiro-Wilk in order to verify the normality of the data and

analyze the variance, means for the treatments were compared by the Tukey test, at 5% probability of error. Because they did not follow the normality assumptions, the variables of stomatal density for the abaxial and adaxial portion, and chlorophyll *a*, *b*, total and carotenoids were transformed via \sqrt{x} . The graphics were presented with non-transformed averages. All statistical analyses were performed using the statistical program SAS (SAS institute Inc, 2010).

Results

Stomatal density

For the stomatal density of the adaxial and abaxial portions of the leaf, by analysis of variance, it was possible to observe that they varied according to the types of environments and cultivars separately at 5% level of significance for F-test.

It was possible to observe a greater stomatal density of the abaxial and adaxial portion in the environment without a net, and lower values for the environment with the silver net ($p < 0.05$) (Figure 2A). In relation to the different cultivars, Vanda presented greater stomatal density in the adaxial and abaxial portions when compared to Vera and Solaris ($p < 0.05$) (Figure 2B).

Stomatal morphometry

For the variable of stomatal morphometry for adaxial and abaxial leaf measurements (length, width and area), it was

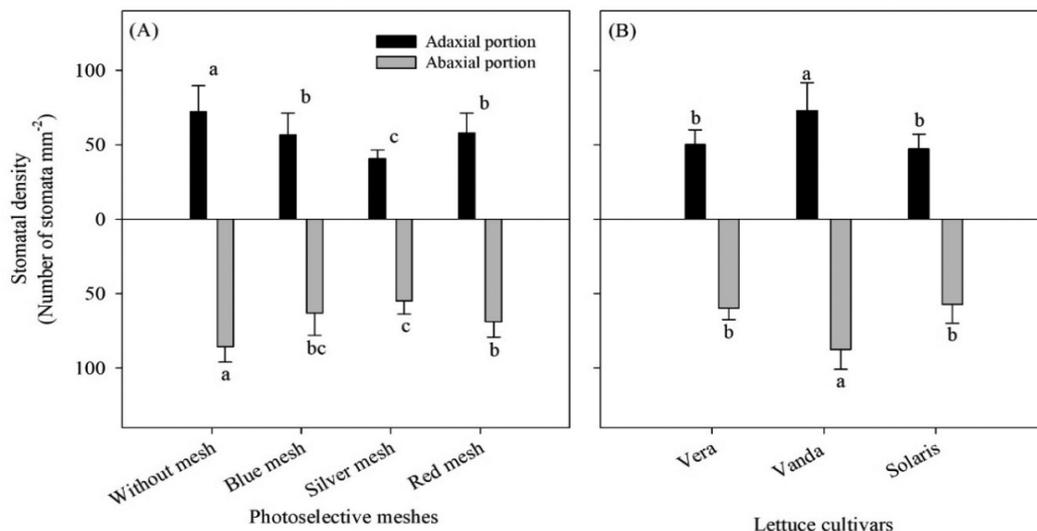


Fig. 2. Stomatal density of the adaxial and abaxial portions of lettuce cultivars (Vera, Vanda e Solaris) under different photoselective nets (Chromatinet® blue, Aluminet® silver, Chromatinet® red and without a net (control), in the winter season

*Means followed by the same letter do not differ by Tukey test at 5% significance

possible to observe interaction between the types of environments x cultivars at 5% probability

When comparing the cultivars within each photosensitive net, it was possible to observe that within the control environment (no net), the cultivar Solaris was superior when compared to the other cultivars for all variables of the adaxial portion of the leaf; for the abaxial portion, and there was no significant difference between treatments or the control.

For the blue net, only the variables of width and area for the abaxial portion, for the cultivar Vera were superior the others ($p < 0.05$). For the other variables, there were no significant differences. For the silver net and the Vera cultivar, superior values were observed for the variables length, width and area of the abaxial portion; and for the cultivar Solaris, the variables length, width and area of the adaxial portion were seen to be superior.

For the red net, it was observed that the cultivar Solaris was superior for all variables analyzed, both in the adaxial portion than abaxial demonstrating greater plasticity of this cultivar when compared to the others ($p < 0.05$). When comparing the different nets within each cultivar, it was observed that the three cultivars presented greater length, width and stomatal area of the abaxial and adaxial leaf portions when grown in a non-net environment. The Vera cultivar presented more ellipsoid stomata in the majority of tested environments for the adaxial side of the leaf. For the abaxial side, the

cultivar Solaris showed a stronger relation between length and width of the stomata (Table 1).

Photosynthetic pigments

For the analysis of variance, photosynthetic pigment variables (chlorophyll *a*, *b*, total and carotenoids) differed for the interaction of net factors x cultivars at 5% probability.

When evaluating cultivars within each type of environment, it was possible to observe that Vanda was significantly superior to other cultivars when cultivated in all environments (chlorophyll *a*, *b*, total and carotenoids). When evaluating the effect of environments on a cultivar, for the variable chlorophyll *a* and total chlorophyll, the cultivar Vera cultivated under the blue net was superior except when cultivated under red net. For the cultivar Vanda, there was no significant difference between the control without a net and the red net treatment. For the cultivar Solaris, there were no significant differences (Table 2).

For the variable chlorophyll *b*, the Vera cultivar did not differ significantly when grown under different types of environments. The cultivar Vanda, when cultivated without net, was significantly superior only when cultivated under the silver net. The cultivar Solaris was observed to have greater chlorophyll *b* content when cultivated under the silver net differing only when grown without a shading net.

Table 1. Morphometry of stomata: length (L- μm), width (W- μm) and area (L*W- μm^2) In the adaxial portion of three lettuce cultivars (Vera, Vanda e Solaris) cultivated under four different photosensitive nets (without a net, blue, silver and red net) during the winter season

Photosensitive nets	Adaxial length (L, μm)			Abaxial length (L, μm)		
	cv. Vera	cv. Vanda	cv. Solaris	cv. Vera	cv. Vanda	cv. Solaris
No net	13.20 ab A	12.20 b A	13.80 a A	13.93 a A	13.33 a A	14.06 a A
Blue	12.33 a AB	12.26 a A	12.73 a BC	13.33 a A	13.00 a A	13.20 a AB
Silver	12.40 ab AB	12.06 b A	13.26 a AB	13.20 a A	11.26 b B	12.13 b C
Red	11.46 a B	8.80 b B	11.93 a C	12.60 a A	10.86 b B	12.60 a BC
Photosensitive nets	Adaxial width (W, μm)			Abaxial width (W, μm)		
	cv. Vera	cv. Vanda	cv. Solaris	cv. Vera	cv. Vanda	cv. Solaris
No net	7.93 b B	9.60 a A	9.86 a A	8.60 a A	8.26 a A	8.06 a AB
Blue	9.13 a A	9.20 a AB	9.66 a AB	9.40 a A	8.26 b A	8.06 b AB
Silver	8.66 ab AB	8.46 b B	9.40 a AB	9.13 a A	7.46 b B	7.93 b B
Red	8.13 ab b	7.33 b C	8.80 a B	7.33 b B	6.86 b B	8.86 a A
Photosensitive nets	Adaxial area (L*W, μm^2)			Abaxial area (L*W, μm^2)		
	cv. Vera	cv. Vanda	cv. Solaris	cv. Vera	cv. Vanda	cv. Solaris
No net	104.6 b AB	118.20 b A	137.86 a A	121.86 a A	110.60 a A	114.06 a A
Blue	112.86 a A	112.80 a A	124.00 a A	125.53 a A	107.80 b A	106.86 b AB
Silver	106.66 b AB	102.33 b A	124.80 a A	117.33 a A	83.46 b B	95.46 b B
Red	91.13 aB	65.86 b B	103.93 a B	91.26 b B	75.60 c B	111.40 a A

*Means followed by the same lowercase letters in the column do not differ the cultivars within the same environment. The same capital letters do not differentiate the cultivar in the different environments, by the test of Tukey to 5% of significance

Table 2. Chlorophyll *a*, *b*, total *e* carotenoids (mg g⁻¹ Fresh phytomass) of the lettuce cultivars (Vera, Vanda and Solaris) grown under different photoselective nets (no net, blue, silver and red net) in the winter season

Photoselective nets	Chlorophyll <i>a</i> (mg g ⁻¹ Fresh phytomass)			Chlorophyll <i>b</i> (mg g ⁻¹ Fresh phytomass)		
	cv. Vera	cv. Vanda	cv. Solaris	cv. Vera	cv. Vanda	cv. Solaris
No nets	0.3168 ab B	0.4963 a A	0.3494 a B	0.0598 a B	0.0949 a A	0.0622 b B
Blue	0.3388 a B	0.4414 ab A	0.324 a B	0.0637 a B	0.0829 ab A	0.0699 ab AB
Silver	0.3138 ab B	0.3976 b A	0.3236 a B	0.0603 a B	0.0795 b A	0.08 a A
Red	0.2723 b C	0.4852 a A	0.3612 a B	0.0517 a C	0.0936 ab A	0.0722 ab B
Photoselective nets	Total Chlorophyll (mg g ⁻¹ Fresh phytomass)			Carotenoids (mg g ⁻¹ Fresh phytomass)		
	cv. Vera	cv. Vanda	cv. Solaris	cv. Vera	cv. Vanda	cv. Solaris
No nets	0.3766 ab B	0.5913 a A	0.4113 a B	0.1334 a C	0.208 a A	0.1528 a B
Blue	0.4025 a B	0.5243 ab A	0.3939 a B	0.133 a B	0.1812 bc A	0.1283 b B
Silver	0.374 ab B	0.4771 b A	0.4037 a B	0.1288a B	0.1613 c A	0.1318 ab B
Red	0.324 b C	0.5789 a A	0.4334 a B	0.1129 a C	0.2017 bc A	0.1471 ab B

*Means followed by the same lowercase letters in the column do not differ by cultivar for the different photoselective nets, capital letters do not differ by cultivar within the same type of photoselective nets by the Tukey test at 5% significance

The cultivar Vanda obtained higher levels of pigment when compared to Vera and Solaris, which could be an inherent characteristic of the cultivar. The Vera cultivar presented a lesser quantity of photosynthetic pigments under red net when compared to the other environments.

As for the variable of carotenoid content, the Vera cultivar did not present a significant difference; however, for the Vanda cultivar, the control treatment (no net) was significantly higher when compared to the other environments. For the cultivar Solaris, it was observed that when grown without net, responses were only significantly greater when compared to values from plants cultivated under the blue net (Table 2).

Discussion

Epidermal characteristics of lettuce leaves

In studying the epidermis of lettuce leaves, it was possible to observe that the cells are formed by sinuous anticlinal walls, a characteristic more pronounced in the abaxial side of the leaf, when compared to adaxial. The lower sinuosity of the cell wall may be related to the adaptive characteristics to prevent against excessive water loss (Lima et al., 2006; Farias et al., 2009), or perhaps due to the lower exposure of the abaxial portion of the leaves to solar radiation. The walls of the epidermal cells were thicker and straight in the leaves of the plants grown without a photoselective net and shaded leaves had more sinuous leaves, demonstrating that the environmental conditions can influence the growth and the development of the plants (Santiago et al., 2001).

The stomata of the lettuce leaves present a reniform

shape, being surrounded by a variable number of cells which do not differentiate in shape and size from the other epidermal cells, characteristic of the anomocytic cellular formations (Alquini et al., 2012). In the median leaf area, mean values of 59 and 75 stomata were observed mm⁻² for the adaxial and abaxial sides, respectively; this is a characteristic of amphistomatic leaves, which is to stay, they have presence of stomata on both sides, but with a greater amount on the abaxial portion. The amphistomatic trait may present a way to increase the photosynthetic rate of the plants, as allows for more efficient gas exchange when compared to hypostomatic leaves (Parkhurst, 1978; Elias et al., 2003).

The two sides of a leaflet of lettuce presented rare occurrences of trichomes, and when present, are a type of trichome. Trichomes can represent a morphological adaptation which favors the assimilation of CO₂ as they promote a reduction of leaf temperature in periods of high air temperature and low water availability (Manetas, 2003); the fact that the center of origin of this plant is in a region with a mild climate, may help to explain the low occurrence of trichomes on both sides of lettuce leaves.

Stomatal density

In the present study, the greatest stomatal density was found when leaves were exposed to high levels of solar radiation which is indicative of better control of the stomatal conductance and a reduction of water loss by transpiration (Voltolini & Santos, 2011) but can limit photosynthesis under certain environmental conditions. This characteristic can lead to greater efficiency of gas exchanges in hours generally characterized by greater relative humidity (Rossatto et al.,

2009). Research by Kundu & Tigerstedt (1999) and Castro et al. (2009) showed positive correlations between number of stomata and photosynthetic rate, both being factors positively correlated with increased crop productivity.

It was also possible to observe that the stomatal density on the two sides was lower in the silver net. These results corroborate with those found by Silva et al. (2015), in which a similar response to the *Tamarindus indica* culture was found. This can be explained by the fact that this net reflects a large amount of incident solar radiation, in addition to promoting a microclimate with temperatures and more agreeable conditions for the plants thus reducing the transpiration and the quantity of stomata.

Due to the specific combinations of light intensity, spectra, temperature and relative humidity, each photoselective net creates a unique or specific microclimate (Zoratti et al., 2015). The same authors reported changes in air temperature between the nets (white, blue and red, for example), as well as between the hours of the day in order to examine the influence of photosynthetically active radiation (PAR) (Lobo et al., 2013), which can promote specific morphophysiological responses in a cultivar (Shahak et al., 2008).

The cultivar Vanda had greater stomatal density in the adaxial and abaxial sides in the present study, which may be related to the genetic effects of the cultivar, as stomatal density may vary between cultivars of the species. In other words, the quantity, distribution, shape and mobility of the stomatal apparatus are characteristics of each species, which can be altered due to environmental conditions, and can thus vary between different individuals of the same species (Larcher, 2006). Thus, we suggest additional studies regarding the effects of photoselective nets, because genotypes are shown to respond differently to different light conditions (Arthurs et al., 2013).

Stomatal morphometry

The morphology of the leaves demonstrated plasticity, and the characteristics and structures of the leaves were very different under the different applied luminous intensities (Yao et al., 2017). Variations in the size and frequency of the stomata show the ability of plants to rearrange their epidermal structures in response to environmental changes leading to a better performance of stomata in gas exchange and transpiration (Rossatto et al., 2009), and indirectly affecting photosynthesis and therefore the growth of plants. Other authors, Atroch et al. (2001) and Klich (2000) did not find differences in the diameters of the stomata in relation to the different environments, in which *Bauhinia forticata* and *Elaeagnus angustifolia*, were submitted. The morphometry of the stomata provides an important indication as to the functionality

of this epidermal attachment. For example, the greater the length/width ratio, the more ellipsoid is the stomata, which may result in relatively improved functionality (Khan et al., 2002). Variations in functionality show that the anatomical plasticity of lettuce plants can be a function of a growing environment.

Photosynthetic pigments

Light are an important regulator of growth and the development of plants, as plants are able to modulate their morphological characteristics and physiological properties according to qualities of light (Simlat et al., 2016). Leaves are the principle organs of photosynthesis and transpiration and increases in the rate of photosynthesis are correlated with increases in light intensity (Yao et al., 2017). High light intensity can also lead to leaf wilting and reduce chlorophyll content and photosynthetic efficiency (Shirke & Pathre, 2003), resulting in photooxidative damage to foliar tissues (Bergquist et al., 2007). Dispersive photoselective nets for light are interesting tools (Ilić et al., 2015), which can be used to reduce this damage because they have the ability to selectively filter intercepted solar radiation and thus manipulate the spectrum and light intensity, modulating it towards something more ideal for each plant or which specifically promotes a desired physiological response (Zoratti et al., 2015).

Photoselective nets represent a new agro-technological concept, which aims to combine physical protection with differential filtration of solar radiation to specifically promote desired physiological responses (Ilić et al., 2012), as well as influence photomorphogenesis which is regulated by light to promote better plant growth (Tinyane et al., 2013). Thus, changes in chlorophyll biosynthesis, via spectral variations, may provide lead to overall improvements in plant growth and development (Larcher, 2006). Low or high temperature stress can affect the biosynthesis of chlorophyll of a plant; for example, an increase in chlorophyll *a* content may be due to a possible adaptation of antenna complexes to stress conditions (Camejo & Torres, 2001). Thus, the quantification of chlorophyll *a*, *b* and total chlorophyll is important in understanding the response of plants, especially in relation to the photosynthetic activity of plants when submitted to different production environments (Discroll et al., 2006); this is because the concentration of the photosynthetic pigments is indicative of the functional state of the photosynthetic apparatus of a plant (Alvarez et al., 2012).

Lobos et al. (2013), working with blueberries under the photoselective nets observed a reduction of light intensity and plant temperature which increased the photosynthetic efficiency. This was also observed by Ilić et al. (2015), which reported that the chlorophyll content and the photosynthetic

capacity increased in tomatoes as the degree of shading of the crop increased. In the present work, however, when the plants were grown under conditions without a net and with red net, chlorophyll content was superior, except for the cultivar Vera, which did not present high growth indexes when grown under a red photosensitive net.

In the present work, when the cultivars Vanda and Solaris were cultivated without a net, an increase of carotenoids was observed. Ilić et al. (2015) also observed lower levels of carotenoids when tomato plants were produced under silver nets and without a net. Gonçalves et al. (2003) observed elevated carotenoid content in environments of direct sun for the plants *Swietenia macrophylla* and *Dipteryx odorata*.

Photosensitive nets can protect cultivars against excess energy and damage caused by excitation and reactive oxygen species (ROS), which are formed during the process of photosynthesis (Bergquist et al., 2007) and which can lead to a reduction in photosynthetic rate (Simlat et al., 2016).

Conclusion

When submitted to environments without a net and with a red net, lettuce plants presented greater relative stomatal densities and chlorophyll content. The concentration of photosynthetic pigments (chlorophyll and carotenoid content) presented greater variation as a function of a cultivar's response than as one of environmental conditions which would demonstrate that the genotypes responded differently to the environments.

The results of the present study provide data related to the use of photosensitive nets in the production of lettuce cultivars. Additional studies are needed to better understand the physiological mechanisms behind the response of these cultivars to environmental conditions.

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References

- Alquini, Y., Bona, C., Boeger, M. R. T.; Costa, C. G. & Barros, C. F. (2012). Epidermis. In: Appezzato-da-Glória, B.; Carmello-Guerreiro, S. M. (eds), *Plant Anatomy*, Universidade Federal de Viçosa, Viçosa, Minas Gerais, 85-104. (Por)
- Alvarez, C., Sáez, P., Sáez, K., Sánchez-Olate, M. & Ríos, D. (2012). Effects of light and ventilation on physiological parameters during *in vitro* acclimatization of *Gevuina avellana* mol. *Plant Cell, Tissue and Organ Culture*, 110(1), 93-101.
- Alvares, C. A., Stape, J. L., Sentelhas, P. C., de Moraes Gonçalves, J. L. & Sparovek, G. (2013). Koppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22(6), 711-728.
- Arsovski, A. A., Galstyan, A., Guseman, J. M. & Nemhauser, J. L. (2012). Photomorphogenesis. *The Arabidopsis Book*, 10, e0147.
- Arthurs, S. P., Stamps, R. H. & Giglia, F. F. (2013). Environmental modification inside photosensitive shadehouses. *Hortscience*, 48(8), 975-979.
- Atroch, E. M. A. C., Soares, A. M., Alvarenga, A. A. & Castro, E. M. (2001). Growth, chlorophyll content, biomass distribution and anatomical characteristics of young plants of *Bauhinia forficata* Link submitted to different shading conditions. *Ciência e Agrotecnologia*, 25, 853-862 (Por).
- Bergquist, S. Å. M., Gertsson, U. E., Nordmark, L. Y. G. & Olsson, M. E. (2007). Ascorbic acid, carotenoids, and visual quality of baby spinach as affected by shade netting and postharvest storage. *Journal of Agricultural and Food Chemistry*, 55(21), 8444-8451.
- Braga, F. T., Pasqual, M., Castro, E. M., Dignart, S. L., Biagiotti, G. & Porto, J. M. P. (2009). Quality of light on the *in vitro* culture of *Dendranthema grandiflorum* cv. Raga: morphophysiological characteristics *Ciência e Agrotecnologia*, 33(2), 502-508 (Por).
- Camejo, D. & Torres, W. (2001). High temperature effect on tomato *Lycopersicon esculentum* pigment and protein content and cellular viability. *Cultivos Tropicales*, 22(3), 13-17.
- Campos, M. L., Almeida, M., Rossi, M. L., Martinelli, A. P., Litholdo Jr, C. G., Figueira, A., Rampelotti-Ferreira, F. T., Vendramim, J. D., Benedito, V. A. & Peres, L. E. P. (2009). Brassinosteroids interact negatively with jasmonates in the formation of anti-herbivory traits in tomato. *Journal of Experimental Botany*, 60(15), 4347-4361.
- Casierra-Posada, F., Zapata-Casierra, E. & Chaparro-Chaparro, D. A. (2014). Growth analysis in chard plants *Beta vulgaris* L. Cicla, cv. 'Pencas Blancas' exposed to different light quality. *Agronomía Colombiana*, 32(2), 205-212.
- Castro, E. M., Pereira, F. J. & Paiva, R. (2009). Plant histology: structure and function of vegetative organs. *Lavras, UFLA*, 9(4).
- Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L. O., Van Der Horst, G. T., Batschauer, A. & Ahmad, M. (2011). The cryptochromes: blue light photoreceptors in plants and animals. *Annual Review of Plant Biology*, 62, 335-364.
- Cutter, E. (1987). Plant anatomy (Anatomia vegetal). Part 1 - Cells and tissues. São Paulo, Roca (Por).
- Driscoll, S. P., Prins, A., Olmos, E., Kunert, K. J. & Foyer, C. H. (2006). Specification of adaxial and abaxial stomata, epidermal structure and photosynthesis to CO₂ enrichment in maize leaves. *Journal of Experimental Botany*, 57(2), 381-390.
- Elias, S. R. M., Assis, R. M., Stacciarini-Seraphin, E. & Rezende, M. H. (2003). Leaf anatomy in young plants of *Solanum lycocarpum* A.St.-Hil. (Solanaceae). *Brazilian Journal of Botany*,

- 26(2), 169-174 (Por).
- Evans, J. R.** (2013). Improving photosynthesis. *Plant Physiology*, 162(4), 1780-1793.
- Farias, V., Rocha, L. D., Preussler, K. H. & Maranhão, L. T.** (2009). Leaf structural organization of *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum, Myrtaceae. *Acta Botanica Brasilica*, 23, 398-406 (Por).
- Ferreira de Melo Júnior, J. C. & Torres Boeger, M. R.** (2016). Leaf traits and plastic potential of plant species in a light-edaphic gradient from Restinga in Southern Brazil. *Acta Biológica Colombiana*, 21(1), 51-62.
- Franklin, K. A. & Quail, P. H.** (2010). Phytochrome functions in Arabidopsis development. *Journal of Experimental Botany*, 61(1), 11-24.
- Gonçalves, L. A., Barbosa, L. C. A., Azevedo, A. A., Casali, V. W. D. & Nascimento, E. A.** (2003). Production and composition of the essential oil of alfavaquinha *Ocimum selloi* Benth. in response to two levels of solar radiation. *Revista Brasileira de Plantas Mediciniais*, 6(1), 8-14 (Por).
- Gratani, L.** (2014). Plant phenotypic plasticity in response to environmental factors. *Advances in Botany*, 1-17.
- Hiscox, J. D. & Israelstam, G. F.** (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, 57(12), 1332-1334.
- Ilić, Z. S., Milenković, L., Stanojević, L., Cvetković, D. & Fallik, E.** (2012). Effects of the modification of light intensity by color shade nets on yield and quality of tomato fruits. *Scientia Horticulturae*, 139, 90-95.
- Ilić, Z. S., Milenković, L., Šunić, L. & Fallik, E.** (2015). Effect of coloured shade-nets on plant leaf parameters and tomato fruit quality. *Journal of the Science of Food and Agriculture*, 95(13), 2660-2667.
- Khan, P. S. S. V., Kozai, T., Nguyen, Q. T., Kubota, C. & Dhanwan, V.** (2002). Growth and net photosynthetic rates of *Eucalyptus tereticornis* Smith under photomixotrophic and various photoautotrophic micropropagation conditions. *Plant Cell, Tissue and Organ Culture*, 71(2), 141-146.
- Kiang, N. Y., Siefert, J. & Blankenship, R. E.** (2007). Spectral signatures of photosynthesis. I. Review of Earth organisms. *Astrobiology*, 7(1), 222-251.
- Klich, M. G.** (2000). Leaf variations in *Elaeagnus angustifolia* related to environment heterogeneity. *Environmental and Experimental Botany*, 44(3), 171-183.
- Kundu, S. K. & Tigerstedt, P. M. A.** (1999). Variation in net photosynthesis, stomatal characteristics, leaf area and whole plant phytomass production among ten provenances of neem *Azadirachta indica*. *Tree Physiology*, 19(1), 47-52.
- Labouriau, L. G., Oliveira, J. G. & Salgado-Labouriau, M. L.** (1961). Sweating of *Schizolobium parahyba* Vell. Toledo. I. Behavior in the rainy season, in the conditions of Caeté, Minas Gerais, Brasil. *Anais da Academia Brasileira de Ciência*, 33, 237-257. (Por)
- Larcher, W.** (2006). *Plant Ecophysiology (Ecofisiologia vegetal)*. São Carlos, RIMA Artes e Textos (Por).
- Lee, H., Feakins, S. J. & Sternberg, L. da S. L.** (2016). Carbon and hydrogen isotopic effects of stomatal density in *Arabidopsis thaliana*. *Geochimica et Cosmochimica Acta*, 179, 275-286.
- Li, J., Hikosaka, S. & Goto, E.** (2011). Effects of light quality and photosynthetic photon flux on growth and carotenoid pigments in spinach *Spinacia oleracea* L. *Acta Horticulturae*, 907, 105-110.
- Lima Jr, E. de C. L., Alvarenga, A. A. de, Castro, E. M. De, Vieira, C. V. & Barbosa, J. P. R. A. D.** (2006). Physioanatomy traits of leaves in young plants of *Cupania vernalis* camb. subjected to different shading levels. *Revista Árvore*, 30, 33-41 (Por).
- Lobos, G. A., Retamales, J. B., Hancock, J. F., Flore, J. A., Romero-Bravo, S. & Del Pozo, A.** (2013). Productivity and fruit quality of *Vaccinium corymbosum* cv. Elliott under photo-selective shading nets. *Scientia Horticulturae*, 153, 143-149.
- Macedo, A. F., Leal-Costa, M. V., Tavares, E. S., Lage, C. L. S. & Esquibel, M. A.** (2011). The effect of light quality on leaf production and development of *in vitro*-cultured plants of *Alternanthera brasiliana* Kuntze. *Environmental and Experimental Botany*, 70(1), 43-50.
- Manetas, Y.** (2003). The importance of being hairy: the adverse effects of hair removal on stem photosynthesis of *Verbascum speciosum* are due to solar UV-B radiation. *New Phytologist*, 158(3), 503-508.
- Parkhurst, D. F.** (1978). The adaptative significance of stomatal occurrence on one or both surfaces of leaves. *Journal of Ecology*, 66(2), 367-383.
- Pires, M. V., Almeida, A. A. F., Figueiredo, A. L., Gomes, F. P. & Souza, M. M.** (2011). Photosynthetic characteristics of ornamental passion flowers grown under different light intensities. *Photosynthetica*, 49(4), 593-602.
- Pociecha, E., Rapacz, M., Dziurka, M. & Kolasińska, I.** (2016). Mechanisms involved in the regulation of photosynthetic efficiency and carbohydrate partitioning in response to low- and high-temperature flooding triggered in winter rye *Secale cereale* lines with distinct pink snow mold resistances. *Plant Physiology and Biochemistry*, 104, 45-53.
- Rajapakse, N. C. & Shahak, Y.** (2007). Light quality manipulation by horticulture industry. *Annual Plant Reviews: Light and Plant Development*, 30, 290-312.
- Raven, J. A.** (2002). Selection pressures on stomatal evolution. *New Phytologist*, 153(3), 371-386.
- Rizzini, L., Favory, J. J., Cloix, C., Faggionato, D., O'Hara, A., Kaiserli, E., Baumeister, R., Schäfer, E., Nagy, F., Jenkins, G. I. & Ulm, R.** (2011). Perception of UV-B by the *Arabidopsis* UVR8 protein. *Science*, 332(6025), 103-106.
- Rossatto, D. R., Hoffmann, W. A. & Franco, A. C.** (2009). Stomatal characteristics of congeneric pairs of cerrado and gallery forest growing in a transitional region in central Brazil. *Acta Botanica Brasilica*, 23(2), 499-508 (Por).
- Santiago, E. J. A., Pinto, J. E. B. P., Castro, E. M., Lameira, O. A., Conceição, H. E. O. & Gavilanes, M. L.** (2001). Aspects of the leaf anatomy of the long pepper *Piper hispidinervum* C DC. under different lighting conditions. *Ciência e Agrotecnologia*, 25(5), 1035-1042 (Por).
- SAS institute Inc.** (2010). SAS/STAT 9.2.2 User's Guide. Cary, NC: SAS Institute Inc.
- Schöttler, M. A. & Tóth, S. Z.** (2014). Photosynthetic complex stoichiometry dynamics in higher plants: environmental ac-

- climation and photosynthetic flux control. *Frontiers in Plant Science*, 5, 188.
- Shahak, Y., Gal, E., Offir, Y. & Ben-Yakir, D.** (2008). Photosensitive shade netting integrated with greenhouse technologies for improved performance of vegetable and ornamental crops. *Acta Horticulturae*, 797, 75-80.
- Shahak, Y., Gussakovsky, E. E., Gal, E. & Ganelevin, R.** (2004). Colornets: crop protection and light-quality manipulation in one technology. *Acta Horticulturae*, 659, 143-151.
- Shirke, P. A. & Pathre, U. V.** (2003). Diurnal and seasonal changes in photosynthesis and photosystem 2 photochemical efficiency in *Prosopis juliflora* leaves subjected to natural environmental stress. *Photosynthetica*, 41(1), 83-89.
- Silva, R. A. L., Soares, J. D. R., Dias, G. M. G., Pasqual, M., Chagas, E. A. & Gavilanes, M. L.** (2015). Cultivation of tamarind under colored meshes: anatomical leaf plasticity. *Ciência Rural*, 45(2), 238-244 (Por).
- Simlat, M., Ślęzak, P., Moś, M., Warchol, M., Skrzypek, E. & Ptak, A.** (2016). The effect of light quality on seed germination, seedling growth and selected biochemical properties of *Stevia rebaudiana* Bertoni. *Scientia Horticulturae*, 211, 295-304.
- Spinelli, G. M., Snyder, R. L., Sanden, B. L. & Shackel, K. A.** (2016). Water stress causes stomatal closure but does not reduce canopy evapotranspiration in almond. *Agricultural Water Management*, 168, 11-22.
- Stamps, R. H.** (2009). Use of colored shade netting in horticulture. *Hortscience*, 44(2), 239-241.
- Tinyane, P. P., Sivakumar, D. & Soundy, P.** (2013). Influence of photo-selective netting on fruit quality parameters and bioactive compounds in selected tomato cultivars. *Scientia Horticulturae*, 161, 340-349.
- Voltoini, C. H. & Santos, M.** (2011). Variations in leaf morphology of *Aechmea lindenii* E. Morren Baker var. *lindenii* Bromeliaceae under different environmental conditions. *Acta Botanica Brasilica*, 25(1), 2-10 (Por).
- Weyers, J. D. B. & Johansen, L. G.** (1985). Accurate estimation of stomatal aperture from silicone-rubber impressions. *New Phytologist*, 101(1), 109-115.
- Yan, M., Wang, M., Wang, H., Wang, Y. & Zhao, C.** (2014). Effects of light quality on photosynthetic pigment contents and photosynthetic characteristics of peanut seedling leaves. *The Journal of Applied Ecology (Ying yong sheng tai xue bao)*, 25(2), 483-487 (Ch).
- Yao, X. Y., Liu, X. Y., Xu, Z. G. & Jiao, X. L.** (2017). Effects of light intensity on leaf microstructure and growth of rape seedlings cultivated under a combination of red and blue LEDs. *Journal of Integrative Agriculture*, 16(1), 97-105.
- Yu, X., Liu, H., Klejnot, J. & Lin, C.** (2010). The cryptochrome blue light receptors. *The Arabidopsis Book*, 8, e0135.
- Zhang, S., Ma, K. & Chen, L.** (2003). Response of photosynthetic plasticity of *Paeonia suffruticosa* to changed light environments. *Environmental and Experimental Botany*, 49(2), 121-133.
- Zoratti, L., Jaakola, L., Häggman, H. & Giongo, L.** (2015). Modification of sunlight radiation through colored photo-selective nets affects anthocyanin profile in *Vaccinium* spp. Berries. *PLoS One*, 10(8), e0135935.

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