EFFECT OF LIGHT AND SUCROSE ON PHOTOAUTOTROPHIC AND PHOTOMIXOTROPHIC MICROPROPAGATION OF *Physalis angulata*

EFEITO DA LUZ E SACAROSE NA MICROPROPAGAÇÃO FOTOAUTOTRÓFICA E FOTOMIXOTRÓFICA DE Physalis angulata

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ABSTRACT: The biotechnological interest in genus *Physalis* has increased in the last decades, however, there are still few micropropagation studies of this genus. The objective of this study was to evaluate *P. angulata* photoautotrophic and photomixotrophic micropropagation with gas exchange under seven light spectra and five concentrations of sucrose. Lighting were yellow, blue, white, red, green, red + blue LEDs and natural light filtered by mesh. Sucrose concentrations were 0, 7.5, 15, 22.5 and 30 $g.L^{-1}$. Phytotechnical, anatomical features and photopigment contents were evaluated through stem and root segment length, leaf number, leaf area, chlorophyll a and b contents, carotenoids, adaxial epidermis, palisadic and spongy parenchyma and abaxial epidermis. The data were compared by Scott-Knott's mean test and principal components analysis using the R software. Comparing the variables within lighting types, it was observed that only the screen treatment, screen-filtered natural illumination, obtained assessment in all variables. Comparing the levels of sucrose, it was observed that the treatment 15 $g.L^{-1}$ sucrose obtained the highest number of averages with maximum evaluation. It was concluded that the natural light filtered by screen with 50% of shading allowed the photoautotrophic micropropagation of *P. angulata*. Better development results were observed in photomixotrophic micropropagation with 15 $g.L^{-1}$ of sucrose.

KEYWORDS: Applied botany: Ecological anatomy: Small fruit crops: Plant tissue culture: Photopigments.

INTRODUCTION

The first works on plant tissue culture used a heterotrophic system in which a carbon source was added to the culture medium and the container was sealed with a plastic cap to prevent drying and contamination of the culture medium (MURASHIGE; SKOOG, 1962). Subsequently, work was done using a gas exchange system with the external medium and sugar reduction in the culture medium, which can be classified as photoautotrophic micropropagation (KOZAI, 1991). In addition to normalizing the concentration of the gases inside the tube, the photoautotrophic micropropagation decreases the energy expenditure and the contamination of the culture medium (XIAO; NIU; KOZAI, 2011). For instance, Rocha et al. (2007) achieved a reduction of up to 45% in the expenditure with electricity.

Photoautotrophic micropropagation helps the acclimatization phase, improving the anatomical and physiological characteristics that allow seedling survival and development (WU; LIN, 2013; MARTINS et al., 2015). The increase of transpiration that is provided by the photoautotrophic micropropagation avoids the hyperhydricity of the explant (KOZAI; KUBOTA; JEONG, 1997).

Gas exchange prevents accumulation of ethylene gas, which causes undesired physiological changes in seedlings (KOZAI; KUBOTA, 2001). Micropropagation without gas exchange can trigger physiological and morphological abnormalities such as drastic reduction of the cuticle and formation of nonfunctional stomata, causing higher mortality of the explants (SALDANHA et al., 2012; CHANDRA et al., 2010).

Despite the advantages, the difficulty for implantation of the photoautotrophic micropropagation is the high cost of the mechanized systems and of the porous membranes. One of the most expensive systems is bioreactors, which allow gas exchange and renewal of the culture medium in an automated way (GEORGIEV; EIBL; ZHONG, 2013). Moreira et al. (2013) obtained the best results of shoot and root growth of *C. walkeriana* with the

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use of bioreactors, compared to the traditional and natural ventilation systems. Silva et al. (2007) observed temporary immersion bioreactor systems, with seedlings immersed every 2 hours for 3 min, provided higher number, height and dry matter mass of pineapple sprouts, and the authors concluded that this was the best method for large-scale pineapple micropropagation. Commercial membranes such as MilliSeal®, which has been successfully used in orchid micropropagation (SILVA, 2013), are currently on the market. However, other researchers have successfully evaluated the use of low-cost, hand-crafted porous lids such as cotton swabs (SALDANHA et al., 2012).

Sucrose also interferes with micropropagation with gas exchange. It is called photoautotrophic micropropagation when there is a total absence of sucrose in the culture medium, however when sucrose is added there is photomixotrophic micropropagation (HOANG et al., 2017). According to Couto et al. (2014), the presence of sucrose in the culture medium may inhibit the photosynthetic assimilation of CO_2. However, Fuentes et al. (2005) state that the presence of exogenous sucrose may increase plant survival and growth during the acclimatization phase. Hoang et al. (2017) compared the photoautotrophic and photomixotrophic micropropagation of *Wasabia japonica* and observed a better development of this species under photoautotrophic micropropagation. An explanation for these results would be that sucrose is a signal for various plant metabolic processes, for example, exogenous excess sucrose causes the same stress in the seedlings as the high intensity of light and cold (TOGNETTI, 2013; BADR; ANGERS; DESJARDINS, 2011).

Another challenge of photoautotrophic micropropagation would be to establish the best type of lighting. Plants need light to regulate their development, physiology and morphogenesis (HUCHÉ-THÉLIER et al., 2016). In growth rooms, lighting is provided only by fluorescent lamps or light emitting diodes (LEDs) (BIAN; YANG; LIU, 2014). Among these two options, LEDs have advantages such as high efficiency in the process of light generation, low heat production and availability at different well-defined wavelengths (YEH; CHUNG, 2009). The photoautotrophic micropropagation with the use of natural light is a technique little explored in plant tissue culture, which has great potential for drastic reduction of seedling production costs (ERIG, SCHUCH, 2005). Silva et al. (2012) observed the best in vitro growth from rooting in natural light under greenhouse conditions and the same conditions provided higher leaf thickness and higher stomatal frequency compared to conventional micropropagation.

The genus *Physalis* (Solanaceae) is a taxonomic group easily recognized due to the peculiar morphology of the fruiting, which is characterized by the presence of an additive and inflated fruiting chalice, which expands completely enveloping the fruit (SOARES et al., 2009). The plants of this genus are widely used in the world cuisine, in the form of bittersweet salads, jellies, pies, cakes, juices, seasonings and sauces (PUENTE et al., 2011). However, there are other applications besides food. A number of studies have been carried out at the biotechnological level with the culture of *Physalis* (BERGIER et al., 2012), including micropropagation (KHAN, BAKHT, 2015).

Considering the excellent results observed for other species, Physalis photoautotrophic micropropagation can be an alternative to save electricity, avoid physiological abnormalities and improve acclimatization. In relation to the best type of lighting in *Physalis*, in a work done ex vitro it was observed that colored shade nets are more efficient with 50% shading and the best wavelength varies with the species (SILVA et al., 2016). With the objective of improving the micropropagation of *Physalis angulata*, the effects of photoautotrophic and photomixotrophic micropropagation were compared using cotton pad as a ventilated cover, under six LED qualities and natural light filtered by shade net black with 50% shading.

MATERIAL AND METHODS

Initial establishment of propagules

Seeds of *Physalis angulata* underwent asepsis in 70% alcohol solutions for 10 min, 50% sodium hypochlorite for 20 min and triple wash in autoclaved distilled water. The seeds were germinated in vitro, in test tubes containing MS medium (MURASHIGE; SKOOG, 1962) supplemented with 30 g L^{-1} sucrose, 5.5 g L^{-1} agar and pH adjusted to 5.7 (± 0,1). The tubes were sealed with plastic film and taken to room growth with photoperiod of 16 h of light, average temperature 25 ± 2 ° C and 35 μmol. m^{-2}. s^{-1} of irradiance supplied by white fluorescent lamps. Every 30 days, the seedlings were harvested until the third subculture.

Experimental procedures

Nodal segments (1-cm long and containing a yarn) were inoculated into test tubes containing MS medium (MURASHIGE; SKOOG, 1962)

supplemented with five different concentrations of sucrose (0; 7.5; 15; 22.5; 30 g. L^{-1}). A classic cotton stopper, such as those used to cover aerobic bacterial cultures in tubes, was manufactured by standardizing the cotton mass used at 15 g per stopper. The lighting treatments consisted of screen (natural light filtered by Polysack's Cromatinet® black photoconverter mesh with 50% shading), and six different tubular LED lamps, TEC-LAMP® (TECNAL®) model, at the following wavelengths: blue (450 nm e 77 µmol. m^{-2}. s^{-1}), red (660 nm e 75 µmol. m^{-2}. s^{-1}), blue + red (660 nm + 450 nm e 76 µmol. m^{-2}. s^{-1}), white (Cool White 7.000k e 75 µmol. m^{-2}. s^{-1}), green (525 nm e 74 µmol. m^{-2}. s^{-1}) e yellow (590 nm e 74 µmol. m^{-2}. s^{-1}).

After 30 days of *in vitro* culture the following analises where performed:

- Gas exchanges: The percent concentrations of O_2 and CO_2 inside the tubes were measured with the use of PBI Dansensor respirometer. The calibration was performed with air outside the tube, being 21.1% O_2 and 0.1% CO_2. The apparatus has a needle that has been inserted into each tube by drilling the cotton cap.

- Phytotechnical characteristics: The length of nodal segments, stem and root was measured. The total leaf area was also evaluated using a desktop scanner and ImageJ software version 1.49p.

- Content of photosynthetic pigments: The chlorophyll content test was based on a methodology without leaf maceration according to the methodology of Macedo, Araújo and Castro (2013). Leaf discs of 0.2 g were deposited in capped test tubes containing 10 mL of 80% (v / v) acetone. The tubes containing the leaf discs were stored for 24 h at 5 ° C in the dark. After the incubation described above, a colorimetric analysis was performed in a spectrophotometer. Absorbance reading at wavelengths 645, 652, 663 and 470 nm was performed. The chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid contents were calculated as follows: chlorophyll a = [(12.7 * A663 - 2.69 * A645) V] / 1000W; chlorophyll b = [(22.9 * A 645 - 4.68 * A 663) V] / 1000W; total chlorophyll = [A652 * 1000 * V / 1000W] / 34.5; total carotenoids = [(1000 * A 470-3.27 * chl b) / 229] / (1000 * W). Where: A = absorbance at the indicated wavelength; V = final volume of the chlorophyll - acetone extract; W = fresh matter in grams of the plant material used. With these results the ratio between chlorophylls a and b was also calculated. The results were expressed as milligram of pigment per gram of fresh leaf tissue weight (mg g^{-1}).

- Leaf anatomy: Rectangular fragments of the middle region of the second leaf were placed in F.A.A.70% (JOHANSEN, 1940) for a period of 72 h at 10 ° C. Then the fragments were submitted to dehydration in ethanolic series of concentrations of 70%, 80%, 90% and 100% for 2h each at 10 ° C. The first step of resin infiltration in the samples was performed with a 1: 1 solution of 100% ethanol and liquid methacrylate resin per 24 hours. The second stage of the infiltration was performed with pure and activated resin for 72 h at 10 ° C and the preparation of blocks containing the material with the use of activated resin solution and hardener in an oven at 37 ° C until solidified. Cross sections of 10 µm thickness were performed with semi-automatic microtome. Samples were stained with 0.05% toluidine blue in acetate buffer pH 4.7 for 10 min. The histological sections were photographed with a Canon A630 digital camera coupled to the Olympus model BX 60 microscope. ImageJ software was used to measure adaxial epidermis, palisade parenchyma, spongy parenchyma, abaxial epidermis and leaf thickness. Three measurements were performed per repetition.

2.3 Statistical procedures

The experiment was installed in a completely randomized design and factorial scheme 7 x 5, being 7 lighting treatments and 5 sucrose concentrations. The results of the Scott-Knott test were obtained through the development of scripts in the R (CORE TEAM 2017) software for public use through the package for public use through the ExpDes.pt package version 1.1.2 (FERREIRA et al., 2013). For the analysis of main components, the results were obtained through the development of scripts in the software R (CORE TEAM, 2017) for public use through the package MVar.pt version 1.9.8 (OSSANI; CIRILLO, 2017).

RESULTS

Contamination was not observed in any of the treatments.

By ANOVA (Table 1), Lighting and Sucrose interaction was significant for all variables except CO_2. In addition, all variables, except CO_2, were affected by isolated sources of variation, Lighting and Sucrose (Table 1).

Table 1. ANOVA summary in micropropagated *P. angulata* with gas exchange, treated in factorial scheme 7 (types of illumination) x 5 (concentrations of sucrose).

S.V.	D. F.	Mean Squares and Significances									
		O_2 (%)		CO_2 (%)		Segment (cm)		Stem (cm)		Root (cm)	
Lighting	6	0,0626	*	0,0010	ns	5,69	*	88,80	*	55,58	*
Sucrose	4	0,2139	*	0,0027	ns	46,56	*	1.210,71	*	324,58	*
Lighting X Sucrose	24	0,0118	*	0,0008	ns	1,92	*	29,85	*	41,33	*
Erro	70	0,0057	-	0,0013	-	0,04	-	1,97	-	0,30	-
C.V. (%)	-	0,36		36,17		6,65		10,15		6,68	

S.V.	D. F.	Mean Squares and Significances									
		Leaf Number		Leaf Area (mm^2)		Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Total Chlorophyll (mg/g)	
Lighting	6	17,33	*	7,90E+13	*	2,58	*	0,8185	*	6,20	*
Sucrose	4	133,21	*	5,42E+14	*	1,68	*	0,7881	*	4,61	*
Lighting X Sucrose	24	4,04	*	1,74E+13	*	0,46	*	0,1053	*	0,97	*
Erro	70	0,59	-	7,79E+12	-	0,03	-	0,0099	-	0,06	-
C.V. (%)	-	15,08		28,33		32,94		23,42		26,91	

S.V.	D. F.	Mean Squares and Significances									
		Chlorophyll a/b		Total Carotenoids (mg/g)		Adaxial (µm)		Palisade (µm)		Spongy (µm)	
Lighting	6	1,65	*	0,0061	*	234,12	*	990,40	*	1.548,90	*
Sucrose	4	3,25	*	0,0075	*	1.040,06	*	4.003,80	*	11.698,00	*
Lighting X Sucrose	24	0,31	*	0,0011	*	115,78	*	311,40	*	1.124,70	*
Erro	70	0,02	-	0,0001	-	3,02	-	8,40	-	23,40	-
C.V. (%)	-	16,37		27,7100		10,56		8,97		8,88	

S.V.	D. F.	Mean Squares and Significances			
		Abaxial (µm)		Leaf Thickness (µm)	
Lighting	6	54,84	*	4.470,00	*
Sucrose	4	930,55	*	45.816,00	*
Lighting X Sucrose	24	54,50	*	3.857,00	*
Erro	70	5,02	-	21,00	-
C.V. (%)	-	15,49		4,23	

Means followed by equal letters in the column do not differ significantly from each other by the Scott-Knott test at the 5% level. UFLA, Lavras, MG, Brazil, 2017.
S.V. = source of variation, D. F. = degrees of freedom, C.V. = coefficient of variation, ns = não significativo, * significativo a 5% de probabilidade, ** significativo a 1% de probabilidade

By the hierarchical clustering algorithm, ScottKnott, comparing the variables within Sucrose Content (Table 2), there was gas exchange with the external environment, 21.1% O_2 and 0.1% CO_2, in all treatments. Focusing on the phytotechnical characteristics of Table 2: the largest segment growth was observed in the treatment 30 Sucrose; the highest steam growth was observed from treatments Sucrose 15 to 30; there was no difference in root growth; the treatment with 0 Sucrose presented the smallest number of leaves; and the largest leaf area was observed from the treatments Sucrose 15 to 30. Focusing on the content of photosynthetic pigments of Table 2 (Chlorophyll a, Chlorophyll b, Total Chlorophyll, Chlorophyll a/b and Total Carotenoids), all variables were higher in treatment 15 Sucrose. Focusing on leaf anatomy analysis: the largest adaxial epidermis thickness was observed in the treatment 0; the smallest palisadic and spongy parenchyma were observed in Sucrose 30; the smallest abaxial epidermis was observed in Sucrose 15; and the largest Leaf Thickness was observed in 0 Sucrose.

Table 2. Scott-Knott's test for the means of the analyzed variables in micropropagated *P. angulata* with gas exchange, treated in factorial scheme 7 (types of illumination) x 5 (concentrations of sucrose).

Sucrose g.L^{-1}	O_2 (%)	CO_2 (%)	Segment (cm)	Stem (cm)	Root (cm)
0	21,10 b	0,10 a	1,50 d	5,53 b	9,47 a
7,5	21,10 b	0,10 a	2,60 c	9,70 b	8,53 a
15	21,17 b	0,10 a	4,20 b	20,50 a	9,67 a
22,5	21,40 a	0,07 a	3,97 b	21,30 a	10,10 a
30	21,40 a	0,10 a	5,47 a	20,43 a	10,50 a
CV (%)	0,49	55,33	5,30	22,07	6,93

Sucrose	Leaf Number	Leaf Area (mm^2)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
0	4,67 b	76.618,67 b	1,22 b	0,88 b	2,10 b
7,5	6,00 a	94.491,67 b	1,12 b	0,80 b	1,92 b
15	6,67 a	144.971,67 a	2,91 a	1,58 a	4,48 a
22,5	6,33 a	160.128,00 a	0,79 b	0,62 b	1,41 b
30	6,67 a	142.396,67 a	0,93 b	0,69 b	1,62 b
CV (%)	13,46	21,55	19,88	20,69	18,81

Sucrose	Chlorophyll a/b	Total Carotenoids (mg/g)	Adaxial (µm)	Palisade (µm)	Spongy (µm)
0	1,36 b	0,07 b	28,86 a	57,64 a	57,64 a
7,5	1,42 b	0,07 b	18,27 c	52,91 a	52,91 a
15	1,85 a	0,16 a	13,07 d	50,29 a	50,29 a
22,5	1,31 b	0,05 b	22,74 b	47,09 a	47,09 a
30	1,37 b	0,06 b	26,87 a	34,84 b	34,84b
CV (%)	15,93	24,88	5,32	7,73	9,29

Sucrose	Abaxial (µm)	Leaf Thickness (µm)
0	18,10 a	185,51 a
7,5	17,83 a	155,36 b
15	11,89 b	134,08 c
22,5	19,61 a	149,74 b
30	20,91 a	108,02 d
CV (%)	11,90	11,90

Means followed by equal letters in the column do not differ significantly from each other by the Scott-Knott test at the 5% level. UFLA, Lavras, MG, Brazil, 2017.

In Figure 1, the photograph of the seedlings shows that all treatments have developed except treatments 0 Sucrose in combination with artificial lighting treatments, LEDs. That is, in the treatment

0 sucrose, only the natural light of the greenhouse (GH) allowed to obtain samples. The Lighting treatments YEL and BLU showed lower development in all Sucrose combinations (Figure 1). The treatment GRE showed lower development in combination 15 and 22,5 Sucrose (Figure 1).

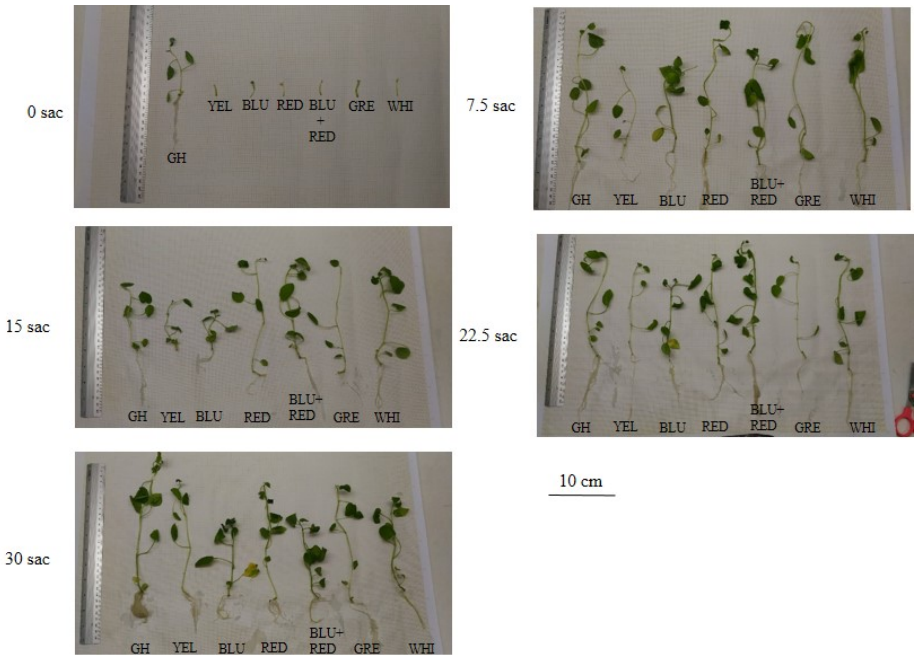

Figure 1. *P. angulata* seedlings micropropagated with gas exchange and treated in factorial scheme 7 (types of illumination) x 5 (sugar concentrations).
Caption: sac = sucrose; SCR = mesh filtered natural light with 50% shading; YEL = Yellow LED; BLUE = Blue LED; RED = Red LED; BLUE + RED = 30% Blue LED + 70% Red; GRE = Green LED; WHI = White LED. UFLA, Lavras, MG, Brazil, 2017.

By the ScottKnott (Table 3), comparing the variables within Lighting Variation (Table 3), there was gas exchange through the caps, O_2 and CO_2. Focusing on the phytotechnical characteristics of Table 3: the largest segments were observed in the treatments Red and Screen; treatments did not affect stem growth; the biggest rooting occurred in the treatments Green, Red, Screen and White; the largest number of leaves and leaf área occurred in treatments Blue, Blue + Red, Screen and White. Focusing on the content of photosynthetic pigments of Table 3: the highest contents of Chlorophyll a, b, Total Chlorophyll, ratio Chlorophyll a/b and Total Carotenoids were obtained in combination with natural light in Screen. Focusing on leaf anatomy analysis: the greatest development of adaxial epidermis was observed in Screen and Yellow; the largest development of the palisadic and spongy parenchyma was observed in Screen; and no significant difference was observed in the Abaxial epidermis and Leaf Thickness.

From the photographs of the anatomical sections of leaves (Figure 2), the material presented good conservation condition. The cell walls, although very thin, remained intact. The thickness of the cuts allowed the individualization of the tissues without overlapping cells. The trichomes appeared sparingly, thinner and more delicate than the rest of the material. As expected, no bacterial and fungal contamination was observed. A little artifact of staining technique was observed, but did not disturb the accurate measurements. The treatments with blue LED and natural light (Screen) presented a bigger and better developed palisade parenchyma (Figure 2).

By Principal Component Analysis (Table 4), specifically Component 1, Leaf Number, ratio Chlorophyll a/b, Palisade, Spongy, Abaxial and Leaf Thickness were strongly correlated (≥ 0,800). For the first component, the variables Segment, Stem, Root, Leaf Area, Chlorophyll b, Total Chlorophyll, Total Carotenoids and Adaxial showed moderate correlation. The Second Component showed no relevant correlation among the variables analyzed in this experiment.

Table 3. Test of means of analyzed variables in *P. angulata* micropropagated with gas exchange, treated with five different concentrations of sucrose, focusing only on the Lighting variation.

Lighting	O_2 (%)	CO_2 (%)	Segment (cm)	Stem (cm)	Root (cm)
Blue	21,09 b	0,09 a	2,16 b	11,04 a	6,18 b
Blue + Red	21,09 b	0,11 a	2,65 b	13,52 a	7,33 b
Green	21,17 a	0,10 a	2,97 b	15,21 a	8,62 a
Red	21,12 b	0,11 a	3,78 a	17,73 a	9,96 a
Screen	21,23 a	0,09 a	3,55 a	15,49 a	9,65 a
White	21,13 b	0,11 a	2,73 b	11,95 a	10,12 a
Yellow	21,03 b	0,09 a	2,23 b	11,78 a	5,31 b
CV (%)	0,59	35,24	53,99	55,17	59,45

Lighting	Leaf Number	Leaf Area (mm^2)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
Blue	5,47 a	106.006,93 a	0,43 b	0,30 b	0,73 b
Blue + Red	6,20 a	117.143,47 a	0,42 b	0,37 b	0,79 b
Green	3,93 b	82.393,47 b	0,33 b	0,40 b	0,73 b
Red	4,73 b	92.489,13 b	0,14 b	0,21 b	0,35 b
Screen	6,07 a	123.721,33 a	1,39 a	0,91 a	2,31 a
White	5,80 a	110.413,27 a	0,45 b	0,49 b	0,94 b
Yellow	3,47 b	57.460,20 b	0,25 b	0,29 b	0,54 b
CV (%)	51,36	57,28	91,78	60,01	75,12

Lighting	Chlorophyll a/b	Total Carotenoids (mg/g)	Adaxial (µm)	Palisade (µm)	Spongy (µm)
Blue	1,07 b	0,03 b	13,91 b	30,81 b	51,66 b
Blue + Red	0,83 c	0,03 b	14,17 b	35,08 b	53,48 b
Green	0,63 c	0,03 b	16,65 b	30,15 b	48,37 b
Red	0,52 c	0,02 b	13,72 b	29,15 b	54,91 b
Screen	1,46 a	0,08 a	21,96 a	48,55 a	75,61 a
White	0,63 c	0,03 b	12,79 b	30,77 b	43,24 b
Yellow	0,66 c	0,02 b	21,99 a	22,04 b	53,98 b
CV (%)	56,90	71,10	51,91	48,43	50,94

Lighting	Abaxial (µm)	Leaf Thickness (µm)
Blue	14,95 a	108,47 a
Blue + Red	13,00 a	110,17 a
Green	14,25 a	100,66 a
Red	13,97 a	102,57 a
Screen	17,67 a	146,54 a
White	11,73 a	93,18 a
Yellow	15,70 a	104,84 a
CV (%)	51,23	48,59

Averages followed by equal letters in the column do not differ significantly from each other by the Scott-Knott test at the 5% level. UFLA, Lavras, MG, Brazil, 2017.

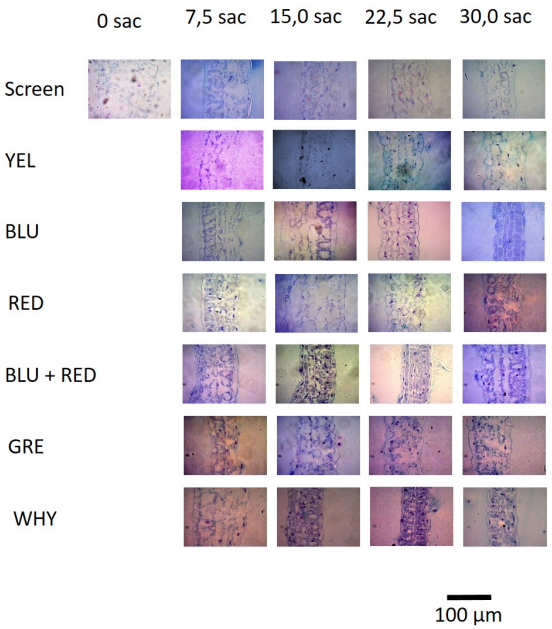

Figure 2. Cross sections of *P. angulata* leaves, micropopayments under gas exchange, and treated in factorial scheme 7 (Lighting) x 5 (Sucrose).
Subtitle: sac = sucrose; SCR = Mesh filtered natural light with 50% shading; YEL = yellow LED; BLUE = blue LED; RED = red LED; BLUE + RED = 30% Blue LED + 70% Red; GRE = green LED; WHI = white LED. UFLA, Lavras, MG, Brazil, 2017.

Table 4. Correlation of the main components with the original variables for the means of the analyzed variables in micropropagated *P. angulata* with gas exchange, treated in factorial scheme 7 (types of illumination) x 5 (concentrations of sucrose).

Comp.	O_2 (%)	CO_2 (%)	Segment (cm)	Stem (cm)	Root (cm)	Leaf Number
1	0,550	-0,227	0,786	0,774	0,706	0,835
2	-0,315	0,181	-0,382	-0,435	-0,313	-0,262

Comp.	Leaf Area (mm^2)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Chlorophyll a/b (mg/g)	Total Carotenoids (mg/g)
1	0,761	0,660	0,792	0,720	0,839	0,760
2	-0,289	0,731	0,532	0,670	0,352	0,622

Comp.	Adaxial (µm)	Palisade (µm)	Spongy (µm)	Abaxial (µm)	Leaf Thickness (µm)
1	0,773	0,925	0,895	0,843	0,923
2	-0,277	0,025	-0,093	-0,297	-0,129

UFLA, Lavras, MG, Brazil, 2017.

In the Analysis of Multiple Factors (Table 5), specifically by the First Componet, Screen showed low correlation (≤ 0,800) with the other treatments, which in turn had a high correlation (≥ 0,800) with each other. The Second Component showed no relevant correlation among the variables analyzed in this experiment.

Table 5. Inertial values computed in the first three axes for each group of observations in *P. angulata* micropropagated with gas exchange, treated in factorial scheme 7 (types of illumination) x 5 (concentrations of sucrose).

Components	Lighting Groups						
	Blue	Blue + Red	Green	Red	Screen	White	Yellow
1	0,913	0,927	0,948	0,962	0,660	0,894	0,966
2	0,209	0,119	0,098	0,099	0,891	0,092	0,170

UFLA, Lavras, MG, Brazil, 2017.

DISCUSSION

Cotton Caps and content of O_2/CO_2

The cotton caps were efficient in allowing gas exchange between the interior of the tubes and the external environment (Table 1, 2 and 3). The O_2 and CO_2 contents in all treatments remained similar to that observed in the calibration, 21.1% O_2 and 0.1% CO_2 (Table 2 and 3). The drastic reduction of CO_2 is observed in heterotrophic micropropagation, with sealed tube (ERIG; SCHUCH, 2005). In addition, the high concentration of sucrose as a carbon source strongly inhibits photosynthesis, reducing O_2 concentration (ARIGITA; GONZÁLEZ; TAMÉS, 2002). As observed in the Multiple Factor Analysis (Table 4), there was no correlation between O_2 and CO_2. This result is expected because the tubes allowed the normalization of these gases contents with the external environment (Table 1, 2 and 3). In addition, 28 of the 29 surviving treatments Figure 1) contained sucrose in the culture medium, which interferes with photosynthesis (COUTO et al., 2014). Because of these interferences in photosynthesis and gas exchange with the external environment, the $CO_2/0_2$ ratio has not been thoroughly analyzed, despite its importance in photorespiration, biomass production and metabolites (VANCE; SPALDING, 2005). In addition, we did not observe any contamination with microorganisms through the cotton lids. Despite the great performance presented by the cotton caps, the system has not been tested on large scale.

Sucrose effect on *Physalis angulata* micropropagation

In 0 Sucrose treatments, the luminous intensity of the LEDs was not sufficient to stimulate photosynthesis to the point of promoting the development of the axillary buds of the nodal segments (Figure 1). Clearly, a totally photoautotrophic micropropagation (0 Sucrose) occurred only under natural light filtered by the mesh with 50% shading (treatment Screen). All other treatments containing sucrose developed seedlings, even if small in some cases (Figure 1). The analysis of multiple factors clearly showed this performance difference (inertia 0.660 by compomponet 1) between the Screen treatment and the LED treatments (Table 5). If no LED light quality was able to develop seedlings under 0 Sucrose, the problem was luminous quantity, ie photon flux rate. The low photon flux rate of the lamps used in the growth chamber is a challenge known to researchers in the area (KOZAI; NGUYEN, 2003). The electric energy expenditure is a great obstacle to try to increase the quantity or power of the lamps (ERIG; SCHUCH, 2005).

The presence of sucrose promoted the best performance of photopigments at a concentration of 15 mg / L, treatment 15 Sucrose (Table 2). McCarthy et al. (2016) also found higher chlorophyll production in Arabidopsis micropropagated at this sucrose concentration, while higher concentrations inhibited photosynthesis.

From the leaf anatomical point of view, sucrose decreased the leaf thickness, probably due to the reduction of the palisade and spongy parenchyma (Table 2). The effect of sucrose is very important for the correct development of plant cells and tissues (MUKHERJEE et al., 2019). It was observed that the 0 Sucrose treatment had a greater thickness of palisade parenchyma, spongy and foliar thickness in relation to the other treatments (Table 2). Comparing the effect of sucrose concentrations within the screen treatment, it was observed that 0 (zero) sucrose presented a greater thickness of palisade and spongy parenchyma than the treatment (30) sucrose (Table 3). This result indicates that excess sucrose in the culture medium can inhibit the formation of the major tissues responsible for photosynthesis (CHANDRA et al., 2010; SALDANHA et al., 2012). This is one of the reasons why there is a decrease in the photosynthetic assimilation of CO_2 in the presence of sucrose in the culture medium (COUTO et al., 2014). These results also indicate that stress is present during the formation of seedlings due to excess sucrose (TOGNETTI, 2013; BADR; ANGERS; DESJARDINS., 2011).

Lighting effect on *Physalis angulata* micropropagation

Comparing the effect of illumination on seedling development, Red and Screen promoted higher segment growth (Table 3). Screen also promoted greater rooting, along with Red, Green and White (Table 3). The higher rooting even under natural light, Screen treatment, corroborates the results of Nunes et al. (2018) that observed that *Physalis* is photoblastic positive. The higher rooting under Red and Green Light corroborates the theory that the absence of blue light improves rooting. Kurilčik et al. (2008) also observed that blue light inhibited root formation. Blue light is currently known to inhibit root development of *Arabdopsis thaliana* which is mediated by different classes of flavin-based photoreceptors (CHRISTIE et al., 2015). Blue light has been observed to increase the production of reactive oxygen species (ROS) in the root meristem (YOKAWA et al., 2013) and it is known that ROS is involved in the control of cell division (LIVANOS et al., 2012). Height growth without the support of other beneficial traits may mean that seedlings are under stress by shading (NOZUE et al., 2015). But no treatment had features that indicated shadow avoidance syndrome.

Greater Number of Leaves and Leaf Area were observed in the Blue, Blue + Red, Screen and White treatments (Table 3). De Sousa, et al. (2019) didn't observe differences in the effect of different lightings on leaf number and size of *P. angulata* micropropagated under different LEDs. This difference in outcome is clearly due to the fact that these authors (DE SOUZA et al., 2019) performed only photomixotrophic micropropagation, whereas in our experiment there was gas exchange and a sucrose content gradient.

The Screen Treatment promoted the best photosynthetic pigment production (Table 3). The greater content of photopigments mean greater efficiency in light energy capitation, defense against stress and prevention against stress (HANNOUFA; HOSSAIN, 2012).

The lighting effect is very important for the correct development of plant cells and tissues (MUKHERJEE et al., 2019). In all surviving seedling treatments of this work (Figure 2), there were one layer of adaxial epidermis, one layer of palisade parenchyma perpendicular to the epidermis, three to four layers of spongy parenchyma with irregularly shaped cells, bicolateral bicuspid vascular bundles and one layer of abaxial epidermis. Therefore, these treatments provided tissue layers similar to that observed in field-grown *Physalis* (THEPSITHAR; THONGPUKDEE, 2013). Screen promoted the best development of the palisadic and spongy parenchyma (Table 3). Screen also promoted the greatest thickness of the adaxial epidermis, along with the Yellow LED treatment (Table 3). Therefore, Screen treatment presented the most similar results to field cultivated *P. angulata* (THEPSITHAR; THONGPUKDEE, 2013). These results are very important for the seedling acclimatization phase.

Correlation Analysis between the analyzed variables

In the analysis of main components (Table 4), there was a strong correlation between variables important for light capture and photosynthesis, being leaf number, chlorophyll a / b, palisade, spongy, abaxial and leaf thickness. Larger leaf number may be an indicator of higher photosynthetic capacity (LI et al., 2015) and better physiological state (VAARIO et al., 2011). The greater chlorophyll a / b ratio, in turn, indicates the greater efficiency of light absorption. Chlorophyll a is more efficient for photosynthesis, while chlorophyll b is more efficient for light uptake (STREIT et al., 2005). The decrease of the chlorophyll a / b ratio indicates that the seedling is undergoing shading and is investing in the antenna complex to try to increase the light capitation (LICHTHENHALER et al., 2007). In this experiment, the palisade variable always remained with a layer of cells (Figure 2), therefore, the increase of this tissue indicated an increase in the cell area. The increase of the cell area of the palisade parenchyma indicates better chloroplast rearrangement for better light uptake, avoiding overlap (OGUCHI; HIKOSAKA; HIROSE, 2003). In addition, cell enlargement may provide better contact and transport between cells (CANNY; HUANG, 2006). Similar to the palisade parenchyma, the number of cells in the spongy parenchyma did not change (Figure 2), so the differences in thickness of this tissue are due to the growth of cells or cell spaces. The increase of the cells of the spongy parenchyma may indicate a greater accumulation of salts and nutrients (SORIN et al., 2015). On the other hand, the increase in cell space in the spongy parenchyma indicates greater metabolic activity and gas exchange (TERASHIMA et al., 2011). The same can be said for leaf thickness. The increase in the thickness of the abaxial epidermis (Figure 2) indicates a greater development of trichomes, which in turn are directly involved in the process of gas exchange and temperature maintenance for photosynthesis and respiration (WANG et al., 2013).

Much remains to be studied for the genus Physalis to have its full biotechnological potential explored. But interest in this genus has grown in recent years because of its enormous pharmacological potential (AM; NIDALVANI, 2014; SHARMA et al, 2015). Because of this potential, the genus Physalis is emerging as a model plant for genetic and molecular biology studies (GARZÓN-MARTÍNEZ et al., 2012; ZHANG et al., 2014). We believe that this work will contribute to the biotechnological exploration of Physalis angulata, through the knowledge of its in vitro ecological behavior under the studied conditions.

CONCLUSIONS

The treatment 0 Sucrose in combination with Screen promoted the photoautotrophic micropropagation of *P. angulata*.

The LED treatments didn't promote photoautotrophic micropropagation of *P. angulata*.

RESUMO: O interesse biotecnológico em Physalis aumentou nas últimas décadas, porém, ainda existem poucos trabalhos de micropropagação desse gênero. Objetivou-se avaliar sua micropropagação fotoautotrófica e fotomixotrófica com troca gasosa sob sete tipos de iluminação e cinco concentrações de sacarose. Foram utilizados LEDs amarelo, azul, branco, vermelho, verde, vermelho + azul e luz natural filtrada por malha. As concentrações de sacarose foram 0, 7,5, 15, 22,5 e 30 $g.L^{-1}$. Características fitotécnicas, anatômicas e teor de fotopigmentos foram avaliados através de comprimento de segmento de caule e raíz, número de folhas, área foliar, teores de clorofilas a e b, carotenoides, epiderme adaxial, parênquimas paliçádico e esponjoso e epiderme abaxial. Os dados foram comparados por teste de média Scott-Knott e análise de componentes principais utilizando-se o software R. Comparando-se as variáveis dentro de tipos de iluminação, observou-se que apenas o tratamento screen, iluminação natural filtrada por malha, obteve avaliação máxima em todas as variáveis. Comparando-se os níveis de sucrose, observou-se que o tratamento 15 $g.L^{-1}$ sacarose obteve o maior número de médias com avaliação máxima. Concluiu-se que a luz natural filtrada por tela com 50% de sombreamento permitiu a micropropagação fotoautotrófica de *P. angulata*. Observou-se melhores resultados de desenvolvimento na micropropagação fotomixotrófica com 15 g.L-1 de sacarose.

PALAVRAS-CHAVE: Botânica aplicada. Anatomia ecológica. Pequenas frutas. Cultura de tecidos vegetais. Fotopigmentos.

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