GUSTAVO COSTA SANTOS

GAS EXCHANGE AND INDUCTION OF POLYPLOIDS IN VITRO IN SPECIES OF GENUS *Physalis*

LAVRAS – MG
2017

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Botânica Aplicada, área de concentração produção vegetal, para a obtenção do título de Doutor.

Prof. Dr. Moacir Pasqual
Orientador

Profa. Dra. Joyce Dória Rodrigues Soares
Coorientadora

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À CAPES, CNPQ e UFLA.

RESUMO GERAL

O gênero *Physalis* é um grupo taxonômico conhecido pelo fruto coberto por um cálice acrescido, saboroso e ornamental. Mais recentemente, esse taxon despertou o interesse biotecnológico devido a produção de fisalinas, uma classe de moléculas com variadas propriedades medicinais. Neste conjunto de trabalhos, a cultura de tecidos, anatomia e citogenética são utilizadas para aumentar o conhecimento biotecnológico sobre algumas espécies de *Physalis*. No primeiro artigo, a qualidade e quantidade de luz emitida pelo LED afetou a características fitotécnicas, anatomia e conteúdo de fotopigmentos de Physalis angulata micropropagadas in vitro. No segundo artigo, qualidade e quantidade de luz também afetaram essas características daquela espécie, porém a micropropagação fotoautotrófica foi obtida usando luz natural e troca gasosa. No terceiro artigo, Physalis alkekengi tetraploides foram obtidas por duplicação cromossômica induzida in vitro e várias características foram alteradas. Em conclusão geral, a cultura de tecidos vegetais contribui significativamente para os avanços biotecnológicos do gênero Physalis.

Palavras-chave: Botânica aplicada. Anatomia vegetal. Cultura vegetal dos tecidos. Genética vegetal. Physalis.

GENERAL ABSTRACT

The genus *Physalis* is a taxonomic group known for the fruit covered by an enlarged chalice, tasty and ornamental. More recently, this taxon has aroused the biotechnological interest due to the production of physalins, a class of molecules with varied medicinal properties. In this set of studies, tissue culture, anatomy and cytogenetics are used to increase biotechnological knowledge on some *Physalis* species. In the first article, the quality and quantity of light emitted by the LED affected the phytotechnical characteristics, anatomy and content of photopigments of in vitro micropropagated *Physalis angulata*. In the second article, quality and quantity of light also affected these characteristics of this species, but the photoautotrophic micropropagation was obtained using natural light and gas exchange. In the third article, *Physalis alkekengi* tetraploides were obtained by in vitro induced chromosome duplication and several characteristics were altered. In general conclusion, plant tissue culture contributes significantly to the biotechnological advances of the *Physalis* genus.

Key words: Applied botany. Vegetal anatomy. Vegetal tissue culture. Plant genetic. Physalis.

SUMMARY

FIRST PART

1 GENERAL INTRODUCTION

Genus *Physalis* L. (*Solanaceae*) is a taxonomic group easily recognized due its peculiar morphology of fruiting, which has presence of an additive and inflated fruiting cup that expands completely enveloping the fruit. *Physalis* diversity estimates between 75 and 90 species. The diversity center of this group is Mexico, where two-thirds of the species are endemic. In South America, they observed approximately 12 species (SOARES et al., 2009).

Plants of this genus are widely used in 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, et al., 2009).

There is growing interest in identifying and developing new genotypes of *Physalis* with different objectives. Polyploidization in plants is a phenomenon that occurs naturally and is important for sympatric speciation (ARVANITIS et al., 2010). Approximately 70% of spermatophytes are tetraploids (ZHANG et al., 2012). Biotechnologically, there are several protocols for polyploidization, and *in vitro* realization is an excellent way to obtain tetraploid clones and to evaluate them always maintaining a copy (OLIVEIRA et al., 2013). Their occurrence may result in characteristics beneficial to the plant such as drought tolerance (MANZANEDA et al., 2012). Polyploidy also leads to changes in the anatomical and phytotechnical characteristics of the plants (TAVAN et al., 2015). In the pharmacological area, polyploidization may increase the production of substances of interest by plants (MAJDI et al., 2010). Science knows that in *P. peruviana*, the degree of ploidy interferes in the resistance to the phytopathogenic fungus of the genus *Fusarium* (LIBERATO et al., 2014; OSORIO-GUARÍN et al., 2016). In *P. ixocarpa*, there are studies comparing the fruit quality of diploid and tetraploid plants (RAMÍREZ-GODINA et al., 2013).

REFERENCES

AFROZ, F. et al. In vitro shoot proliferation and plant regeneration of Physalis minima L. – a perenial medicinal herb. **Bangladesh J. Sci. Ind. Res.**, v. 44, n. 4, p. 453-456, March, 2009.

ARVANITIS L. et al. Novel antagonistic interactions associated with plant polyploidization influence trait selection and habitat preference. **Ecology letters**, v. 13, n. 3, p. 330-337, January, 2010.

BERGIER, K. et al. Antioxidant profiles and selected parameters of primary metabolism in Physalis ixocarpa hairy roots transformed by two Agrobacterium rhizogenes strains. **Acta Biologica Cracoviensia Series Botanica**, v. 54, n. 1, p. 24 – 33, November, 2012.

GARZÓN-MARTÍNEZ, G. A. et al. The Physalis peruviana leaf transcriptome: assembly, annotation and gene model prediction. **BMG Genomics**, v. 13, n. 1, p. 1 – 12, April, 2012.

KHAN, W.; BAKHT, J. Development of efficient and optimized protocol for rapid micropropagation of Physalis ixocarpa, a medicinal herb. **Pak. J. Weed Sci. Res.**, v. 21, n. 3, p. 381 – 391, March, 2015.

LIBERATO, S. et al. Cytogenetic of *Physalis peruviana* L., and *Physalis floridana* Rydb. Genotypes with differential response to *Fusarium oxysporum*. **Corpoica Ciencia y Tecnología Agropecuaria**, v. 15, n. 1, p. 51-61, January, 2014.

MANZANEDA A. J. et al. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). **New phytologist**, v. 193, n. 3, p. 797-805, January, 2012.

OLIVEIRA, S. C. et al. In vitro polyploidization from shoot tips of Jatropha curcas L.: a biodiesel plant. **Plant Growth Regulation**, v. 69, n. 1, p. 79-86, January, 2013.

OSORIO-GUARÍN et al. Association analysis for disease resistance to *Fusarium oxysporum* in cape gooseberry (*Physalis peruviana* L). **BMC Genomics**, v. 17, n. 248, March, 2016.

PUENTE, L. A. et al. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. **Food Research International**, v. 44, n. 7, p. 1733-1740, August, 2011.

RAMAR, K et al. In vitro shoot multiplication and plant regeneration of *Physalis peruviana* L. na importante medicinal plant. **Int. J. Curr. Microbiol. App. Sci.**, v. 3, n. 3, p. 456-464, March, 2014.

RODRIGUES, F. A. et al. Diferentes concentrações de sais do meio MS e BAP na multiplicação in vitro de *Physalis peruviana* L. **Bioscience Journal**, v. 29, p. 77-82, March, 2013.

ROBLEDO-TORRES, V. et al. Development of tomatillo (*Physalis ixocarpa* Brot.) autotetraploids and their chromosome and phenotypic characterization. **Breeding Science**, v. 61, n. 3, p. 288 – 293, June, 2011.

SOARES, E. L. C. et al. O gênero *Physalis* L. (Solanaceae) no Rio Grande do Sul, Brasil. **Pesquisas (Botânica)**, v. 1, n. 60, pp. 323 – 340, Jan., 2009.

TAVAN, M. et al. In vitro polyploidy induction: changes in morphological, anatomical and phytochemical characteristics of *Thymus persicus* (Lamiaceae). **Plant Cell Tiss Organ Cult**, v. 122, n. 3, p. 573-583, May, 2015.

WANG, L. et al. Functional analysis of an iaaM gene in parthenocarpic fruit development in transgenic Physalis pubescens L. plants. Plant Cell, Tissue and Organ Culture, v. 107, n. 2, p. 333-340, November, 2011.

ZHANG, M. et al. Genome physical mapping of polyploids: a BIBAC physical map of cultivated tetraploid cotton, *Gossypium hirsutum* L**. PLoS One**, v. 7, n. 3, p. e33644, March, 2012.

SECOND PART - ARTICLES

Article 1 - In vitro culture of *Physalis angulata*: Effect of light-emitting diodes on the physiological and anatomical characteristics of micropropagated *Physalis angulata*

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ABSTRACT

Interest in the biotechnological potential of *Physalis angulata* has increased due to its pharmacological potential. Little is known about the effect of light quality on the development of micropropagated *Physalis*; therefore, we selected six LED types, namely, blue, blue + red, yellow, green, white, and red, to evaluate their effects on morphological, anatomical, and photopigment characteristics. Yellow, red, and green LED treatments led to greater stem and nodal segment development. Blue, blue + red, and white LEDs resulted in less elongated stems and larger leaf area. Plants treated with blue LEDs had thicker palisade parenchyma and more elongated cells. Thus, we concluded that different LED treatments affected plantlet morphology, foliar anatomy, and foliar photopigment content of micropropagated *P. angulata*. The blue LED treatment generated better morphogenesis and cellular differentiation of photosynthetic tissues.

Key words: Applied botany. Ecological anatomy. Small fruit crops. Vegetal tissue culture. Photopigments.

1 INTRODUCTION

Physalis L. (Solanaceae) is a taxonomic group that has about 120 species and its main characteristic is an inflated calyx, which expands and completely envelopes the fruit (Am and Nidavani, 2014). This small fruit is mainly produced in Colombia and South Africa (Muniz et al., 2014), but the plant is also cultivated in several other countries. Its exploitation occurs mainly in traditional agriculture and medicine, but there is growing biotechnology research

into the therapeutic potential of *Physalis,* such as elimination of the protozoan *Leishmania amazonensis* (SILVA et al., 2016), antibacterial activity (RIVERA et al., 2015), anti-inflammatory activity (SANG-NGERN et al., 2016), blood glucose reduction (DAUD et al., 2016), anti-cancer effect (XU et al., 2016; PENG et al., 2016), and antiulcerogenic effects (RASHEED et al., 2016). *Physalis* biotechnological research includes genetic engineering (Bergier et al., 2012), transcriptomics (GARZÓN-MARTÍNEZ et al., 2012), gene function analysis (WANG et al., 2011), and polyploidization (ROBLEDO-TORRES ET AL., 2011). In the plant tissue culture field, studies have been published on the micropropagation of *P. ixocarpa* (KHAN; BAKHT, 2015), *P. minima* (AFROZ et al., 2009), and *P. peruviana* (RAMAR et al., 2014). Hairy root establishment, in vitro callus induction (MUNGOLE et al., 2011), and in vitro flowering induction in *P. minima* (RAMAR; AYYADURAI, 2015) and synthetic seed production in *P. peruviana* (YÜCESAN et al., 2015) have also been investigated. Physalin production (AZLAN et al., 2002) and extraction of other medicinal compounds from *P. peruviana* callus (GAUTAM et al., 2011, LASHIN; ELHAW, 2016) have been conducted in the field of medicinal biotechnology.

The effect of light on plant phytotechnical and biochemical characteristics is a field of interest in plant biotechnology. Among commercialized light sources, LEDs have advantages such as high efficiency in light generation, low heat production, and availability at different well-defined wavelengths (YEH; CHUNG, 2009). Considering these advantages, NASA began the first research with LEDs for vegetal production in the isolated environment of space stations (BIAN et al., 2014). The effect of different types of LEDs on several plant species in indoor cultivation has been tested (GUPTA; JATOTHU, 2013). In plant tissue culture, there are different objectives such as increased somatic embryogenesis rate (CHEN et al., 2016), growth enhancement and morphogenesis (LI et al., 2013), in vitro induction of flowering (DEWIR et al., 2007) or germination (GODO et al., 2011), and production of secondary compounds (SILVA et al., 2013, CHEN et al., 2016).

In view of the great biotechnological potential of *Physalis* and the lack of knowledge on the effect of artificial light on the micropropagation of this genus, we aimed to investigate the effect of six different LEDs on the in vitro development of *P. angulata*.

2 MATERIALS AND METHODS

2.1 Obtaining plant material

Physalis angulata seeds, obtained in Rio Grande do Sul state Brazil, underwent 10 min 70% alcohol asepsis, 20 min 50% sodium hypochlorite asepsis and were washed four times in autoclaved distilled water. The seeds were in vitro inoculated in test tubes containing MS salts (MURASHIGE; SKOOG, 1962) plus 30 g l^{-1} Sucrose, 5.5 g l^{-1} agar and pH adjusted to 5.7 (± 0.1). The tubes were sealed tubes with plastic film and kept in a growth room with a 16 h photoperiod, 25 ± 2 °C temperature, and 35 µmol m^{-2} s^{-1} irradiance white fluorescent lamps. Every 30 days, the seedlings were micropropagated for three subcultures.

2.2 Experimental procedures

We inoculated nodal segments, which had one axillary bud, into test tubes containing MS medium as described above. Then, we maintained the material under six different light qualities, provided by TEC-LAMP® (TECNAL®) LED tubular lamps. These six lamps had the following luminous wavelength and photosynthetically active radiation rate: blue (450 nm and 77 µmol m^{-2} s^{-1}), red (660 nm and 75 µmol m^{-2} s^{-1}), blue + red (660 nm and 74 µmol m^{-2} s^{-1}), white (cool white 7,000 k and 75 µmol m^{-2} s^{-1}), green (525 nm and 74 µmol m^{-2} s^{-1}) and yellow (590 nm and 74 µmol m^{-2} s^{-1}). We build experimental structure using two tubular lamps per treatment, 50 cm from the base of the test tubes and with 30 cm between lamps. After 30 days, we performed the following analyses:

2.2.1 LEDs photosynthetically active radiation rate estimation using an Infrared Gas Analyzer, IRGA, LI-6400XT System (Li-Cor).

2.2.1.1 Phytotechnical analysis

Using a pachymeter, we measured steam length from base to apical meristem, root length from base to cap, segment 1 length from base to first axillary bud, and segment 2

length from base to second axillary bud. We also counted the leaf number. We evaluated the leaf area of the second-born leaf using a scanner and ImageJ v. 1.49 p software (public domain). We also evaluated the dry matter content, using a forced air circulation oven, desiccator-glass, and precision balance. The experimental design completely randomized with four replicates per treatment.

2.2.1.2 Photopigment content

We assessed chlorophyll content by a non-maceration methodology (Macedo et al., 2013). We placed 0.5 g leaflet fragments in test tubes containing 10 ml 80% (v/v) acetone, which were incubated at 5 °C in the dark for 24 h. After incubation, we transferred the solution to cuvettes for spectrophotometer analysis. The reference sample consisted of 80% (v/v) acetone solution. We performed absorbance readings at 645, 652, 663 and 470 nm wavelengths. Chlorophyll a, chlorophyll b, total carotenoids, and total chlorophyll content were calculated using the following estimates: chlorophyll a = [(12.7 × A663 – 2.69 × A645) V]/1000 × W, Chlorophyll b = [(22.9 × A645 - 4.68 × A663) V]/1000 × W, Total chlorophyll = [A652 × 1000 × V/1000 × W]/34.5, and Total carotenoids = [(1000 × A470 - 3.27 × chlorophyll b)/229]/(1000 × W), where A is the absorbance at the indicated wavelength, Vis the acetone volume in milliliters, and W is the fresh matter in grams of leaf. We expressed the photopigment results as milligrams of pigment per gram of fresh leaf tissue weight (mg g^{-1}). The experimental design was completely randomized with four replicates per treatment.

2.2.1.3 Leaf anatomy

In order to standardize the samples, we used a 25 mm^2 rectangular area of the median region of the leaf of the second node. We placed leaf fragments in FAA 70% fixative (Johansen, 1940) for a period of 72 h at 10 °C. Then, we dehydrated the samples by subjecting them to an ethanolic series of 70%, 80%, 90%, and 100%, for 2 h at each concentration, and at 10 °C. Subsequently, we performed the first step of samples infiltration with a 1: 1 solution (ethanol: liquid methacrylate resin) for 24 h. The second infiltration stage consisted of immersing the samples in activated resin for 72 h at 10 °C. After this period, we

carried out material embedding in activated resin solution and hardener, until material solidification in an oven at 37 °C. We aligned blocks containing samples and fixed in wooden supports. We performed 10 μm thickness cross sections in a semiautomatic microtome. We stained samples with 0.05% toluidine blue in pH 4.7 acetate buffer for 20 min. After excess dye removal and drying, we covered slides with acrylic enamel and cover slip. We photographed leaf sections with a Canon A630 digital camera coupled to an Olympus BX 60 microscope. The obtained images allowed measurements of anatomical characteristics using the ImageJ image analysis software. The experimental design was completely randomized. We photographed three histological sections per treatment, took three measurements per section, and performed four replicates, which totaled thirty-six measurements per treatment.

2.3 Statistical analysis

We installed the experiment in a completely randomized design with six treatments and four replicates. We performed the Tukey test through R software scripts for public use through the ExpDes.pt version 1.1.2 package (FERREIRA et al., 2013). For principal component analysis, we obtained results through the development of scripts in the software R for public use through the package MVar.pt version 1.9.1 (OSSANI; CIRILLO, 2016) and the package BiploGUI version 0.0-7 (GRANGE et al., 2013).

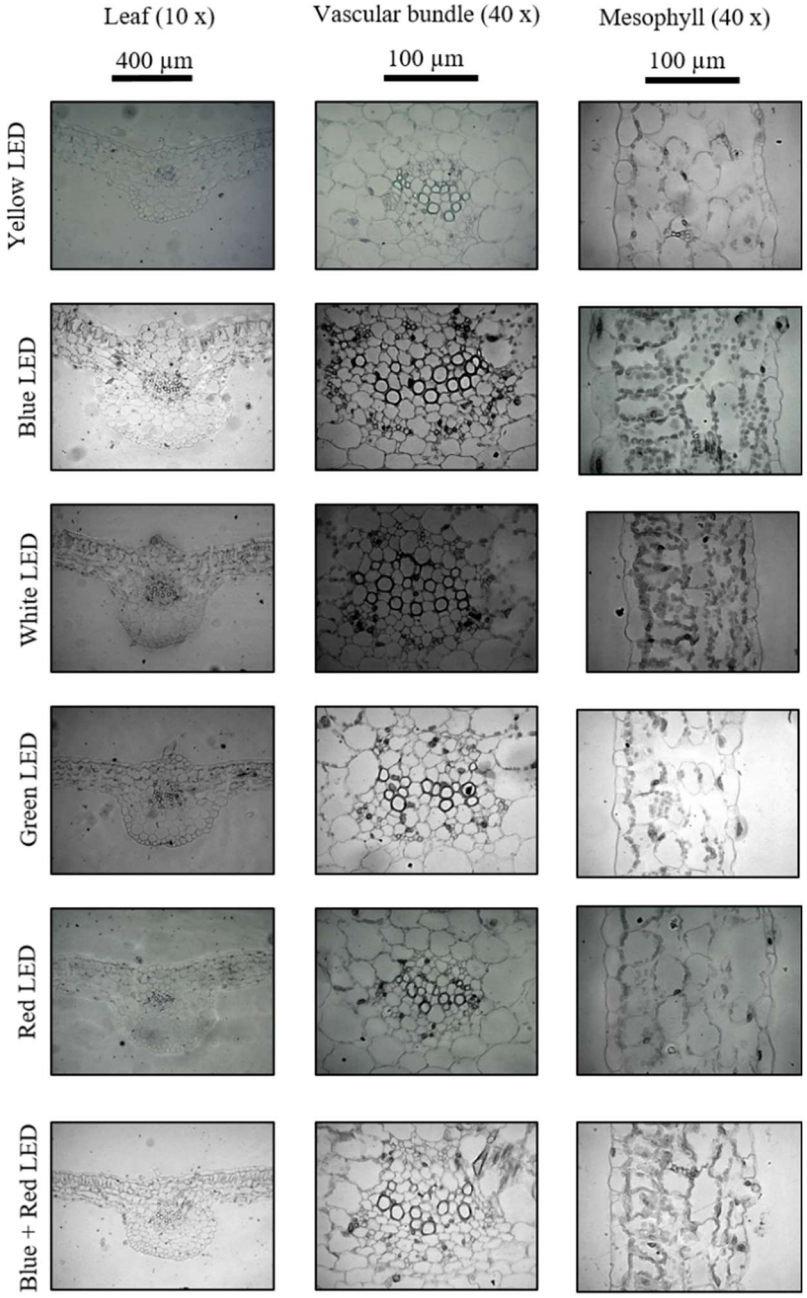
3 RESULTS

We observed greater development of seedlings stem and nodal segment in the treatments with yellow, red, and green LEDs (see FIGURE 1 and TABLE 1). Blue, blue + red, and white LEDs gave less elongated stems, (see FIGURE 1 and TABLE 1). Blue LED treatment resulted in a shorter root length compared to the other treatments. White LED treatment provided a greater leaf number than blue LED, Table 1. Leaf area results showed that white, blue and blue + red LED treatments presented greater leaf area in comparison to the other treatments, (see FIGURE 1 and Table 1).

Figure 1 - *Physalis angulata* seedlings obtained by in vitro propagation under six different LED types. From left to right, seedlings were treated with white, green, blue + red, red, blue, and yellow LEDs. Bar = 2 cm. UFLA, Lavras, MG, Brazil, 2017.

Leaf anatomy followed the same pattern in all treatments: a single layer of adaxial epidermis, a single layer of palisade parenchyma cells, three to four layers of irregularly shaped spongy parenchyma cells, vascular bundle of the bicollateral central vein and a layer of abaxial epidermis, see Figure 2. The cell walls were thin and irregular and had little affinity to the dye.

Figure 2 - Cross section microphotographs of in vitro *Physalis angulata* leaves propagated under different LED qualities. Scales correspond to 50 µm. UFLA, Lavras, MG, Brazil, 2017.



Quantitatively, the blue, blue + red and red LED treatments gave thicker leaf limbs, Table 1. Plants treated with blue LEDs had the thickest palisade parenchyma tissue of all treatments, (see FIGURE 2 and TABLE 1). Qualitatively, we observed that the palisade

parenchyma presented a rectangular shape with blue LEDs, unlike the columnar format observed in the other treatments, Figure 2. We observed no difference in spongy parenchyma and abaxial epidermis, by Tukey test, Table 1. Blue LED treated plants presented thicker adaxial epidermis than green LED treated plants, Table 1. We observed more dry matter in the blue + red LED treatment than in the blue or yellow LED treatments, Table 1.

Table 1 - Tukey test for different variables analyzed in *Physalis angulata* seedlings that were micropropagated under six different LED sources. Averages followed by the same letters in the column do not differ significantly from each other by the Tukey test at the 5% level. UFLA, Lavras, MG, Brazil, 2017.

LED	Segment 1		Segment 2		Stem		Root		Dry matter	
Blue	0,425	d	0,400	c	1,100	d	5,250	b	53,205	b
Blue + red	0,275	d	1,350	bc	3,650	c	7,375	a	125,810	a
Green	1,425	b	4,650	a	11,200	a	8,425	a	83,240	ab
Red	3,125	a	4,900	a	12,125	a	8,000	a	86,795	ab
White	0,900	c	2,625	b	7,000	b	7,675	a	84,220	ab
Yellow	2,925	a	5,825	a	11,300	a	9,400	a	30,875	b
CV(%)	10,77		18,11		13,40		12,11		34,29	

LED	Leaf area		Leaf number		Leaf thickness		Adaxial epidermis		Palisade parenchyma	
Blue	209,076	ab	3,500	b	196,478	a	30,670	a	79,145	a
Blue + red	181,696	bc	4,000	ab	168,788	ab	24,123	ab	57,094	bc
Green	171,950	cd	4,500	ab	153,676	b	19,513	b	48,383	cd
Red	145,019	d	4,500	ab	173,479	ab	26,672	ab	58,888	b
White	220,462	a	5,000	a	150,136	b	25,291	ab	42,656	d
Yellow	100,616	e	3,998	ab	155,391	b	24,396	ab	39,572	d
CV(%)	7,47		14,67		10,53		17,28		7,26	

LED	Spongy parenchyma		Abaxial epidermis		Chlorophyll a		Chlorophyll b		Total chlorophyll	
Blue	86,295	a	18,938	a	0,0530	c	0,0155	b	0,0694	c
Blue + red	80,348	a	16,901	a	0,0583	bc	0,0151	b	0,0744	c
Green	79,608	a	20,835	a	0,0819	bc	0,0252	ab	0,1095	bc
Red	81,373	a	17,902	a	0,0853	bc	0,0270	ab	0,1156	abc
White	73,196	a	17,704	a	0,1284	a	0,0362	a	0,1673	a
Yellow	87,052	a	18,016	a	0,0946	ab	0,0310	a	0,1287	ab
CV(%)	13,35		10,25		21,72		21,70			

LED	Chlorophyll a/b		Chlorophyll/ carotenoids		Total carotenoids	
Blue	3,424	abc	35,469	b	0,0020	c
Blue + red	3,859	a	35,263	b	0,0021	bc
Green	3,217	bc	38,131	ab	0,0029	bc
Red	3,199	bc	38,196	ab	0,0030	abc
White	3,551	ab	39,477	a	0,0042	a
Yellow	3,048	c	38,705	ab	0,0033	ab
CV(%)	6,45		4,32		19,96	

Chlorophyll a, chlorophyll b, and total chlorophyll content of the seedlings did not vary much between the treatments, Table 1, but we observed a greater difference between the white LED and blue LED treatments, Table 1. Pigment light uptake efficiency, represented by the ratio of chlorophyll a/b, was very similar for all treatments, although slightly increased in the plants treated with LEDs with some percentage of blue light, Table 1.

By principal component analysis (PCA), we observed that the first two components accounted for 77.51% of the sample variation, (see TABLE 2 and FIGURE 5) which evidences the analyses could be explained in two-dimensional space. Through observations scores of PCA variables, Figure 3, we observed that the yellow LED treatment was concentrated in the third quadrant, while the other treatments were in the second quadrant, showing that the yellow LED treatment differed from the others. This result could be more clearly observed by Biplot, Figure 4, in which we observed that the yellow LED treatment had little association with the variables, unlike the other treatments. We also observed that blue + red LED treatment was associated with the dry matter variable, blue LED treatment with leaf thickness and palisade parenchyma, and white LED treatment was associated to leaf area.

Table 2 - Variance and proportion of the variance explained by the five main components originated from the six variables (LEDs) in micropropagated *Physalis angulata* seedlings subjected to different LEDs. UFLA, Lavras, MG, Brazil, 2017.

Comp.	Segment 1	Segment 2	Stem	Root	Leaves number	Leaf area
1	-0,653	-0,839	-0,856	-0,861	-0,762	0,441
2	-0,634	-0,498	-0,366	-0,244	0,568	0,788

Comp.	Chlorophyll a	Chlorophyll b	Total chlorophyll	Chlorophyll a/b	Total carotenoids	Chlorophyll /Carotenoids
1	-0,858	-0,928	-0,886	0,541	-0,879	-0,959
2	0,386	0,156	0,319	0,751	0,328	0,062

Comp.	Adaxial epidermis	Palisade parenchyma	Spongy parenchyma	Abaxial epidermis	Leaf thickness	Dry matter
1	0,563	0,896	0,371	-0,091	0,865	0,219
2	-0,090	-0,130	-0,8c96	-0,278	-0,277	0,650

Figure 3 - Projections of two main components originated from the six variables (LEDs) in micropropagated *Physalis angulata* seedlings subjected to different LED treatments. UFLA, Lavras, MG, Brazil, 2017

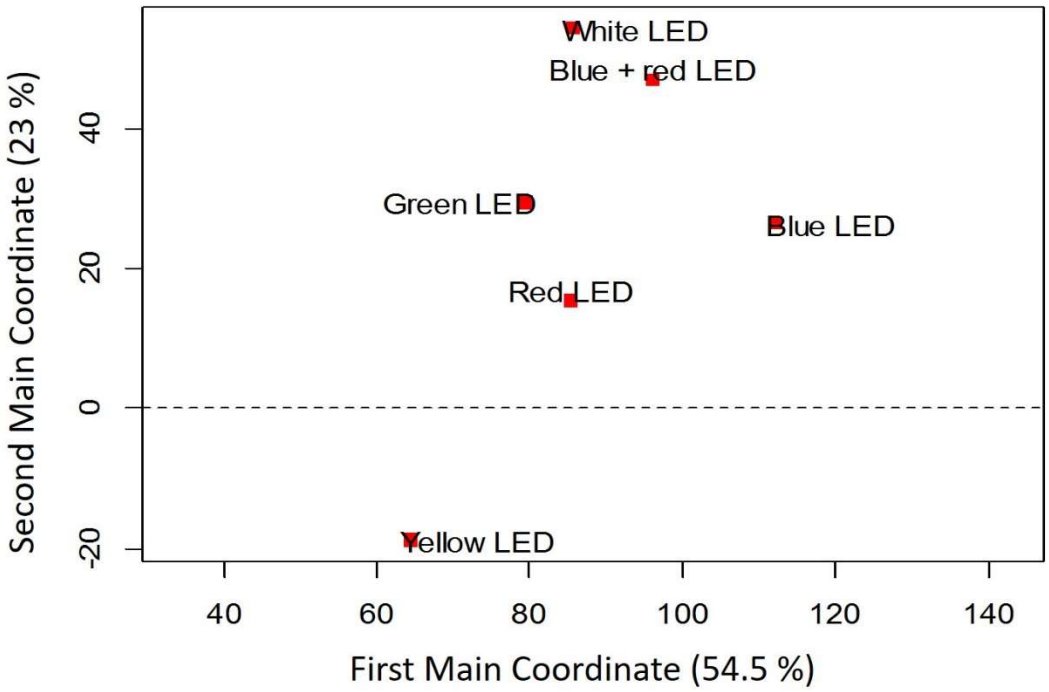


Figure 4 - LED treatments and biological variables associated with micropropagated *Physalis angulata* seedlings subjected to different LED treatments. UFLA, Lavras, MG, Brazil, 2017.

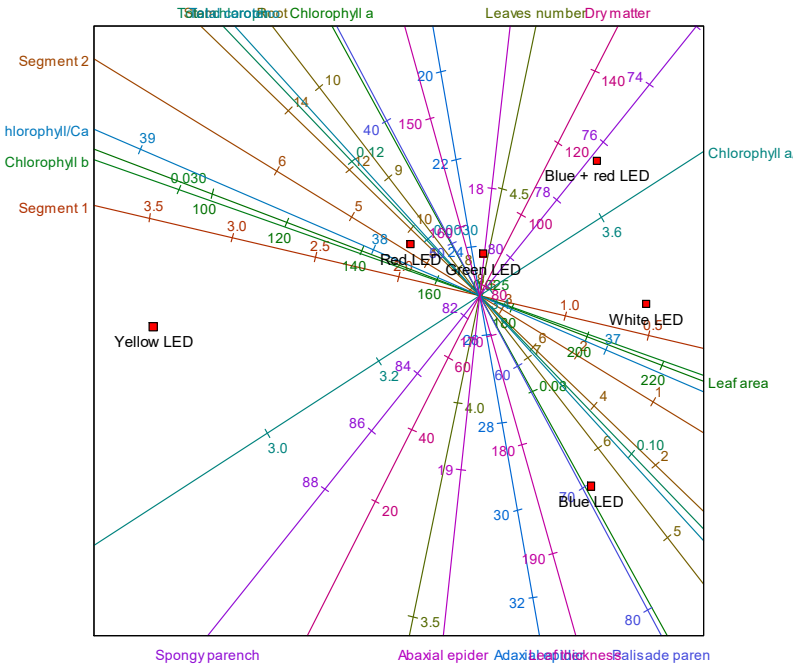
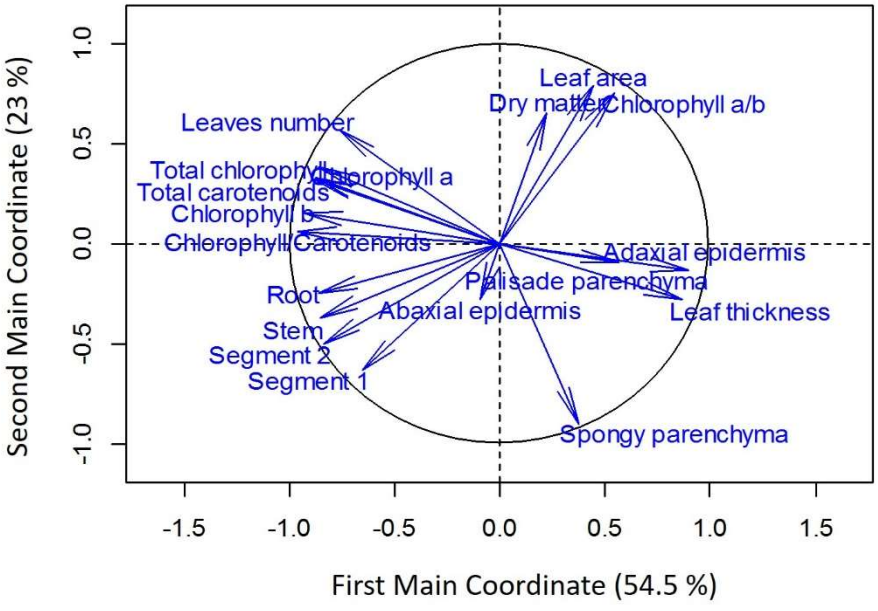


Figure 5 - Correlations between the variables with the main components in micropropagated *Physalis angulata* seedlings subjected to different LED treatments. UFLA, Lavras, MG, Brazil, 2017.



Multiple factor analysis indicated analyses could be explained in a two-dimensional space, since explained variation proportion in the first two components was 72.30% of sample variation, Table 4.

When the partial inertia of each group, interpreted as the amount of variation returned in each component inherent to the global analysis, was analyzed, Table 5, we observed a strong similarity between red, blue + red, green, and white LED treatment groups, relative to the first main component. Regarding the second main component, there was high similarity between the yellow and blue LED treatment groups, Table 5. To better interpret the inertia obtained in each group (TABLE 4) we generated an inertia graph, Figure 6.

Figure 6 - Inertia of the LED groups. UFLA, Lavras, MG, Brazil, 2017.

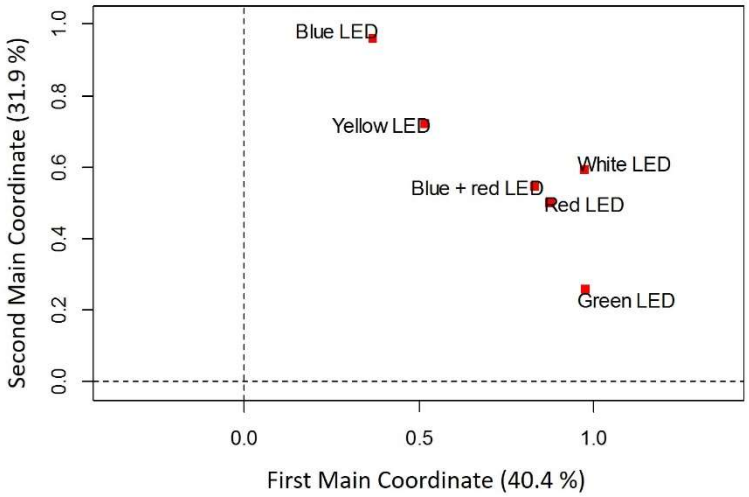


Table 3 - Correlation of the main components with the original variables in micropropagated *Physalis angulata* seedlings subjected to different LED treatments.

Components	Variance	Explanation %	Accumulated %
1	9,810	54,50	54,50
2	4,141	23,00	77,51
3	1,974	10,96	88,47
4	1,354	7,52	95,99
5	0,721	4,01	100,00

Table 4 - Proportion explained by components in micropropagated *Physalis angulata* seedlings subjected to different LED treatments. UFLA, Lavras, MG, Brazil, 2017.

Components	Variance	Explanation %	Accumulated%
1	4,539	40,40	40,40
2	3,583	31,90	72,30
3	3,112	27,70	100,00

Table 5 - Values of inertias computed in the first three axes for each group of observations. UFLA, Lavras, MG, Brazil, 2017.

	LED						
Component	Yellow	Blue	Red	Blue+ Red	Green	White	Total Inertia
1	0,516	0,369	0,875	0,830	0,975	0,973	4,539
2	0,721	0,961	0,502	0,547	0,259	0,593	3,583
3	0,428	0,181	0,997	0,486	0,436	0,583	3,112

4 DISCUSSION

The large stem and nodal segment development observed after red, green, and yellow LED treatments (FIGURE 1 and TABLE 1) is likely because of an adaptive response to canopy shading called 'shade avoidance syndrome' (SAS). According to our hypothesis, *P. angulata*, a sun-loving plant, is able to avoid shading by other plants by detecting (via cryptochromes) reduced blue light that is commonly absorbed by the canopy (PEDMALE et al., 2016). Red, green, and yellow LED treatments have lower blue radiation than that in white, blue, and blue + red LED treatments. It is known that SAS is dependent on increased ethylene content (PIERIK et al., 2009), and this phytohormone readily accumulates in plant tissue culture sealed flasks (BIDDINGTON, 1992). Green light promotes young stem elongation in Arabidopsis (Folta, 2013) and the same effect is likely to have occurred in *Physalis*. The detection of red light (via phytochromes) by plants also promotes stem elongation (Trupkin et al., 2014). Illumination with yellow light results in cryptochrome inactivation, which prevents blue light detection and triggers SAS (Boyd et al., 2007; WANG; FOLTA, 2013).

The root development inhibition observed in blue LED treatment (FIGURE 1, TABLE 1) coincides with the results in other studies (NHUT et al., 2003; KIM et al., 2004; KURILCIK et al., 2008). This indicates that *P. angulata* young roots show negative phototropism. In nature, blue light is the spectrum with the lowest capacity to penetrate soil and reach the root meristem (MO et al., 2015). When negatively phototrophic roots detect blue light, it triggers auxin redistribution and regulates cell divisions in order to inhibit root growth (KUTSCHERA; BRIGGS, 2012). Moreover, studies show that blue light increases the production of reactive oxygen species (ROS) in the root meristem (YOKAWA et al., 2013) and excess ROS halts cell division (LIVANOS et al., 2012).

With respect to *Physalis* leaf biometry, we detected a difference in leaf number between blue and white LED treatments (TABLE 1). We know that light affects leaf number in tomatoes and that it is important for initiating fruiting (UZUN, 2006). However, LED treatment time affects the difference in leaf number as observed in *Hypoestes phyllostachya* (SABAGHNIA et al., 2016). Another leaf biometric parameter analyzed, leaf area, seems to have increased with blue light presence in blue LED, blue + red LED and white LED

treatments (TABLE 1). Blue light breaks apical yolk dominance, redistributing auxins and more nutrients to the leaf (KEUSKAMP et al., 2011). Blue light also increases leaf area in *Lactuca sativa* and *Cucumis sativus* (LIN et al., 2013; WANG et al., 2015). Yellow LED treated plants showed the smallest leaf area (TABLE 1, FIGURE 1), and similar results have been reported in tomato (BRAZAITYTÉ et al., 2010). Regarding yellow LED treatment, we assume that more severe or advanced SAS occurred, affecting both leaves and palisade parenchyma thickness (TABLE 1).

Anatomical leaf structure of micropropagated *P. angulata* (FIGURE 2) was similar to that observed ex vitro by Thepsithar and Thongpukdee (2013) in all LED treatments, indicating that the treatments did not alter cell layer order or number. Cell walls showed low to medium affinity for blue toluidine dye, indicating low lignin content and secondary wall inhibition (O'BRIEN et al., 1964), except in the xylem. Quantitatively, the greater leaf thickness observed in blue LED, blue + red LED, and red LED (TABLE 1) seemed to be associated with higher metabolic, photosynthetic, or respiratory activity (TERASHIMA et al., 2011). According to Oguchi et al. (2003), a small increase in mesophyll thickness may imply that chloroplasts overlap within the cells, contributing little to increasing the photosynthetic rate; therefore, we inferred that the increase in thickness acts favorably in improving photosynthesis. The largest palisade parenchyma in the blue LED treatment was more similar to that described in ex vitro *P. angulata* (THEPSITHAR; THONGPUKDEE, 2013). A similar result has been observed in *Betula pendula* (Sæbø et al., 1995). In all treatments, the spongy parenchyma was approximately 80 μm, as observed for ex vitro *P. angulata* (THEPSITHAR; THONGPUKDEE, 2013).

A small difference in chlorophyll and carotenoid content between treatments (TABLE 1) shows that the effect of LED treatment was lower at the molecular level than at the morphological level. This result may be because, in plant tissue culture, even a low radiation rate is compensated by a greater photoperiod, tube CO_2 accumulation (GUPTA; JATOTHU, 2013), and because the culture medium is a substantial carbon source (MURASHIGE; SKOOG, 1962). This delay in molecular development indicates the need for seedling acclimatization, even though they already have good height (KUMAR; RAO, 2012). The greatest difference in photosynthetic pigment content was between plants subjected to the blue and white LED treatments (TABLE 1). Blue LED treatment resulted in lower

photopigment content, which differs from that observed in other studies where blue light increased photopigment production in other species (SÆBØ et al., 1995; KURILČIK et al., 2008). However, this result did not affect pigment light absorption efficiency, measured by the ratio chlorophyll a/b when comparing blue LED to other treatments (TABLE 1). Regarding the total carotenoid content, we observed that white LED treatment was slightly better, or similar, to the other treatments (TABLE 1). Some studies have shown that white LED treatment increases the expression of genes related to carotenoid production in orchids (Lee et al., 2013). Carotenoid expression can be a leaf senescence indicator (HANNOUFA; HOSSAIN, 2012), but this does not appear to be the physiological state in white LED treatment because it also presented slightly higher light pickup efficiency, represented by the chlorophyll/carotenoid ratio (Table 1). In addition, this result indicates that white LED is the most suitable for in vitro *Physalis* carotenoid production.

Regarding dry matter accumulation in *Physalis seedlings* (TABLE 1), we observed the greatest difference between blue + red and yellow LED treatments; blue + red LED treated plants reached a higher average mass than that by yellow LED treated plants. Most studies have shown that blue and red lights are the light spectra that most stimulate biomass accumulation (BIAN et al., 2014). For this reason, NASA mainly uses these luminous spectra in their micropropagation research (POULET et al., 2014). This combination of two light spectra has a superior efficiency in biomass production as photopigments absorb red light more efficiently, whereas blue light influences stomatal opening and morphology (MASSA et al., 2008). Lower dry matter accumulation in yellow LED treated plants (TABLE 1) was expected because this spectrum ineffectively stimulates photoreceptors (BIAN et al., 2014). However, further studies are required to understand dry matter accumulation in vitro because photosynthesis is not the major limiting factor for in vitro growth, since sucrose is a MS medium ingredient (MURASHIGE; SKOOG, 1962). This is demonstrated by in vitro callus and hairy roots culture grown in the dark (WU ET AL., 2016; KWON et al., 2017).

Principal component analysis showed that yellow LED treatment differed from other treatments (TABLE 2, FIGURE 3, FIGURE 4) probably because this light spectrum least stimulated photosynthesis and phototropism (YEH; CHUNG, 2009). Green light treatment also provided little stimulation for the photoreceptors; however, the results of previous studies indicate that shorter green light wavelengths may provide a greater stimulus (JHOKAN et al.,

2012). In addition, the green light spectrum stimulates phytochemical production by photoreceptor-independent processes (BIAN et al., 2014), which is used by plants for shading perception, for example (WANG; FOLTA, 2013). Blue + red LED treatment was more associated with the dry matter variable (Figure 4), reinforcing the previously discussed idea (TABLE 1) that the light spectra combination is generally the most efficient for bioaccumulation (BIAN et al., 2014). Blue LED treatment is strongly associated with leaf thickness and palisade parenchymal thickness (FIGURE 4), which corroborates the importance of this light spectrum for cellular elongation (FUKUDA et al., 2008). White LED treatment is associated with the leaf area variable (Figure 4) and also had the highest mean leaf area (TABLE 1), which indicate the efficiency of this treatment to avoid at least one SAS symptom in *P. angulata*.

We performed a correlation of the first main component with the original variables, (TABLE 3, FIGURE 5) and observed that Group 1 is composed of variables related to mitosis and synthesis (KUTSCHERA; BRIGGS, 2012), while Group 2 is composed of variables related to cell elongation (KEUSKAMP et al., 2011). A strong inverse correlation between these two groups indicates that *Physalis* seedlings coordinated their physiology by alternating cell multiplication and cell growth in response to the stimuli given by the treatments. We also analyzed the correlation of the second main component with variables (TABLE 3, FIGURE 5) and observed the formation of Group 3 composed of two variables related to SAS. One of these variables, the chlorophyll a/b ratio, decreases in shading because of chlorophyll b investment in the antenna complex, maximizing light absorption (LICHTHENHALER et al., 2007). As with the chlorophyll a/b ratio, leaf area also decreases with shading perception (DAI et al., 2009).

We performed an analysis of multiple factors (TABLE 4) and analyzed partial inertia of each LED group (TABLE 5, FIGURE 6). From the results, we observed that blue and yellow LED treatments differed from other treatments and each other. Comparing these two treatments, we observed that yellow LED was the worst treatment for *Physalis* propagation, because it presented characteristics that were the most similar to SAS, such as elongated stem segments, small leaves, and low chlorophyll a/b ratio (TABLE 1). In turn, blue LED treatment was the best treatment for providing interesting characteristics for acclimatization as leaves and palisade parenchyma were more developed with a shorter stem, which avoids the tilting

of seedlings (TABLE 1). These results indicate a greater potential for blue light morphological stimulus in micropropagated *Physalis* (FUKUDA et al., 2008) although the red light is more absorbable by photopigments (BIAN et al., 2014), probably because the culture medium provides carbon (MURASHIGE; SKOOG, 1962).

5 CONCLUSIONS

Treatments with different LEDs affected morphology, foliar anatomy, and foliar photopigment content of *Physalis angulata* propagated in vitro. The blue LED treatment generated better morphogenesis and cellular differentiation of photosynthetic tissues.

REFERENCES

AFROZ, F.; HASSAN, A.; BARI, L. S.; SULTANA, R.; BEGUM, N.; JAHN, M. A. A.; KHATUN, R *In vitro* shoot proliferation and plant regeneration of *Physalis minima* L.–a perennial medicinal herb. **Bangladesh J Sci Ind Res**, n. 44, p. 453-456, 2009.

AM, M.; NIDAVANI, R. B. *Physalis angulata* L.: An ethanopharmacological review. **Indo Am J Pharm Res,** v. 4, p. 1479-1486, 2014.

AZLAN, G. J.; MARZIAH, M.; RADZALI, M.; JOHARI, R Establishment of *Physalis minima* hairy roots culture for the production of physalins. **Plant Cell Tissue Organ Cult**, v. 69, p. 271-278, 2002.

BERGIER, K.; KUZNIAK, E.; WIELANEK, M.; SKŁODOWSKA, M. Antioxidant profiles and selected parameters of primary metabolism in *Physalis ixocarpa* hairy roots transformed by two *Agrobacterium rhizogenes* strains. **Acta Biol Crac Ser Bot**, v. 54, p. 24-33, 2012.

BIAN, Z. H.; KUŹNIAK, E.; WIELANEK, M.; KRÓLICKA, A.; SKŁODOWSKA, M. Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: a review. **J Sci Food Agric**, v. 95, p. 869-877, 2014.

BIDDINGTON, N. L. The influence of ethylene in plant tissue culture. **Plant Growth Regul**, v. 11, p. 173-187, 1992.

BOULY, J. P.; SCHLEICHER, E.; DIONISIO-SESE, M.; VANDENBUSSCHE, F.; STRAETEN, D. V. D.; BAKRIM, N.; MEIER, B. A.; GALLAND, P.; BITTL, R. et al. Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. **J Biol Chem**, v. 282, p. 9383-9391, 2007.

CHEN, C. C.; AGRAWAL, D. C.; LEE, M. R.; LEE, R. J.; KUO, C. L.; WU, C. R.; TSAY, H. S.; CHANG, H. C Influence od LED light spectra on *in vitro* somatic embryogenesis and LC-MS analysis of chlorogenic acid and rutin in *Peucedanum japonicum* Thunb.: a medicinal herb. **Int J Bot Stud**, v. 57, p. 1-8, 2016.

DAI, Y. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. **Environ Exp Bot**, v. 65, p. 177-182, 2009.

DAUD D.; ELIAS, S.F.; HASSAN, F. S. M.; JALIL, M. N.; TAWANG, A. *Physalis mínima* Linn methanolic extract reduces blood glucose level without compromising sperm quality in normoglycaemic mice. **J Appl Pharm Sci**, v. 6, p. 8-11, 2016.

DEWIR, Y. H.; CHAKRABARTY, D.; HAHN, E. J.; PAEK, K. Y Flowering of *Euphorbia millii* plantlets *in vitro* as affected by paclobutrazol, light emitting diodes (LEDs) and sucrose. **Acta Hort**, v. 764, p. 169-173, 2007.

FERREIRA, E. B.; CAVALCANTI, P.; NOGUEIRA, D. A (2013). D. A. **ExpDes.pt**: Pacote Experimental Designs (Brazilian Portuguese). R package version 1.1.2. Disponível em: <https://cran.r-project.org/web/packages/ExpDes.pt /index.html>. Acesso em: 10 fev. 2017.

FUKUDA, N.; FUJITA, M.; OHTA, Y.; SASE, S.; NISHIMURA, S.; EZURA, H. Directional blue light irradiation triggers epidermal cell elongation of abaxial side resulting in inhibition of leaf epinasty in geranium under red light condition. **Sci Hortic**, v. 115, p. 176-182, 2008.

GAUTAM, Y. K.; RANI, R.; VIMALA, Y. *In vitro* accumulation of active metabolites in *Physalis peruviana* (Linn.) callus. **Vegetos**, v. 24, p. 58-60, 2011.

GARZÓN-MARTÍNEZ, G. A.; ZHU, Z. I.; LANSMAN, D.; BARRERO, L. S.; MARIÑO-RAMÍREZ, L. The *Physalis peruviana* leaf transcriptome: assembly, annotation and gene model prediction. **BMG Genomics,** v. 13, p. 1-12, 2012.

GODO, T.; FUJIWARA, K.; GUAN, K.; MIYOSHI, K. Effects of wavelength of LED-light on *in vitro* asymbiotic germination and seedling growth of *Bletilla ochracea* Schltr. (Orchidaceae). **Plant Biotechnol,** v. 28, p. 397-400, 2011.

GRANGE, A. L.; ROUX, N. **BiploGUI:** Interactive Biplots in R. R package version 0.0-7, 2013. Disponível em: <http://biplotgui.r-forge.r-project.org/>. Acesso em: 10 abr. 2016.

GUPTA, S. D.; JATOTHU, B. Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. **Plant Biotechnol Rep**. v. 7, p. 211-220, 2013.

JOHANSEN, D. A Plant microtechnique. 2nd ed. New York, USA: McGraw-Hill.
JHOKAN, M.; SHOJI, K.; GOTO, F.; HAHIDA, S.; YOSHIHARA, T. Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in *Lactuca sativa*. **Environ Exp Bot**, v. 74, p. 128-133, p. 1940, 2012.

KEUSKAMP, D. H.; SASIDHARAN, R.; VOS, I.; PEETERS, A. J. M.; VOESENEK, L.; PIERIK, R. Blue-light-mediated shade avoidance requires combined auxin and brassinosteroid action in *Arabidopsis* seedlings. **Plant J**, v. 67, p. 208-217, 2011.

KHAN, W.; BAKHT, J. Development of efficient and optimized protocol for rapid micropropagation of *Physalis ixocarpa*, a medicinal herb. **Pak J Weed Sci Res**, v. 21, p. 381-391, 2015.

KUMAR, K.; RAO, I. U. Morphophysiological problems in acclimatization of micropropagated plants in *ex vitro* conditions–A review. **J Ornam Hortic Plants**, v. 4, p. 271-283, 2012.

KURILČIK, A.; MIKLUŠYTĖ-ČANOVA, R.; DAPKŪNIENĖ, S.; ŽILINSKAITĖ, S.; KURILČIK, G.; TAMULAITIS, G.; DUCHOVSKIS, P.; ŽUKAUSKAS, A In vitro culture of *Chrysanthemum* plantlets using light-emitting diodes. **Cent Eur J Biol**, v. 3, p. 161-167, 2008.

KUTSCHERA, U.; BRIGGS, W. R. Root phototropism: from dogma to the mechanism of blue light perception. **Planta,** v. 235, p. 443-452, 2012.

KWON, D. Y.; KIM, H. H.; PARK, J. S.; PARK, S. U.; PARK, N. Production of baicalin, baicalein and wogonin in hairy root culture of American skullcap (*Scutellaria lateriflora*) by auxin treatment. **Biosci Biotech Res Asia**, v. 14, p. 673-677, 2017.

LASHIN, I. I.; ELHAW, M. H. Evaluation of secondary metabolites in callus and tissues of *Physalis peruviana*. **IJMB**, v. 6, p. 10-17, 2016.

LEE, W. L.; HUANG, J. Z.; CHEN, L. C.; TSAI, C. C.; CHEN, F. C. Developmental and LED light source modulation of carotenogenic gene expression in *Oncidium* gower ramsey flowers. **Plant Mol Biol Rep**, v. 31, p. 1433-1445, 2013.

LICHTENHALER, H. K.; AČ, A.; MAREK, M. V.; KALINA. J.; URBAN, O Differences in pigments composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. **Plant Physiol Biochem**, v. 45, p. 577-588. 2007.

LI. H.; TANG, C.; XU, Z. The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis *in vitro*. **Sci Hortic**, v. 150, p. 117-124, 2013.

LIN, K. H.; HUANG, M. Y.; HUANG, W. D.; HSU, M. H.; YANG, Z. W.; YANG, C. M. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. capitata). **Sci Hortic**, v. 150, p. 86-91, 2013.

LIVANOS, P.; APOSTOLAKOS, P.; GALATIS, B. (Plant cell division: ROS homeostasis is required. **Plant Signal Behav**, v. 7, p. 771-778, 2012.

MACEDO, W. R.; ARAÚJO, D. K.; CASTRO, P. R. C Unravelling the physiologic and metabolic action of thiamethoxam on rice plants. **Pest Biochem Physiol**, v. 107, p. 244-249, 2013.

MASSA, G. D.; KIM, H. H.; WHEELER, R. M.; MITCHELL, C. A. Plant productivity in response to LED lighting. **HortScience**, v. 43, p. 1951-1956, 2008.

MO, M.; YOKAWA, K.; WAN, Y.; BALUŠKA, F. How and why do root apices sense light under the soil surface? **Front Plant Sci**, v. 6, 2015.

MUNGOLE, A. J.; DOIFODE, V. D.; KAMBLE, R. B.; CHATURVEDI, A.; ZANWAR, P. *In vitro* callus induction and shoot regeneration in *Physalis minima* L. **Ann Biol Res**. v. 2, p. 79-85, 2011.

MUNIZ, J.; KRETZSCHMAR, A. A.; RUFATO, L.; PELIZZA, T. R.; RUFATO, A. D. R.; MACEDO, T. A. General aspects of *Physalis* cultivation. **Ciênc Rural,** v. 44, p. 964-970, 2014.

MURASHIGE, T.; SKOOG, F. A. A revised medium for rapid growth and bio assays with tobacco tissue cultures. **Physiol Plantarum,** v. 1, p. 473-497, 1962.

O'BRIEN, T. P.; FEDER, N.; MCCULLY, E. Polychromatic staining of plant cell walls by toluidine blue O. **Protoplasma**, v. 59, p. 368-373, 1964.

OGUCHI, R.; HIKOSAKA, K.; HIROSE, T. Does the photosynthetic light-acclimation need change in leaf anatomy? **Plant Cell Environ,** v. 26, p. 505-512, 2003.

OSSANI, P. C.; CIRILLO, M. A (2016). MVar.pt: **Analise multivariada** (Brazilian Portuguese). R package version 1.9.1. Disponível em: <https://cran.r-project.org/web/packages/MVar.pt/index.html>. Acesso em: 13 fev. 2017.

PENG, C. Y.; YOU, B. J.; LEE, C. L.; WU, Y. C.; LIN, W. H.; LU, T. L. Chang FC, Lee HZ The roles of 4β-hydroxywithanolide E from *Physalis peruviana* on the nrf2-anti-oxidant system and the cell cycle in breast cancer cells. **Am J Chin Med**, v. 44, p. 617-636, 2016.

PEDMALE, U. V.; HUANG, S. S. C.; ZANDER, M.; COLE, B. J.; HETZEL, J.; LJUNG, K.; REIS, P. A. B.; SRIDEVI, P.; NITO, K.; NERY, J. R. Cryptochromes Interact directly with PIFs to control plant growth in limiting blue light. **Cell**, v. 164, p. 233-245, 2016.

PIERIK, R.; DJAKOVIC-PETROVIC, T.; KEUSKAMP, D. H.; WIT, M.; VOESENEK, L. A. C. J Auxin and ethylene regulate elongation responses to neighbor proximity signals independent of gibberellin and DELLA proteins in *Arabidopsis*. **Plant Physiol**, v. 149, p 1701-1712, 2009.

POULET, L.; MASSA, G. D.; MORROW, R. C.; BOURGET, C. M.; WHEELER, R. M.; MITCHELL, C. A. Significant reduction in energy for plant-growth light in space using LED light and spectral manipulation. **Life Sci Space Res,** v. 2, p. 43-53, 2014.

RAMAR, K.; AYYADURAI, V *In vitro* direct multiple shoot induction and flower initiation of *Physalis minima* (L.) an important medicinal plant. **JIARM**, v. 3, p. 119-124, 2015.

RAMAR, K.; AYYADURAI, V. ARULPRAKASH, T. *In vitro* shoot multiplication and plant regeneration of *Physalis peruviana* L. an important medicinal plant. **Int J Curr Microbiol App Sci,** v. 3, p. 456-464, 2014.

RASHEED, A.; VARMA, R. K.; KHAN, M.; AKHTAR, A. J. Antisecretory and antiulcerogenic activity of *Physalis angulata* leaves in different experimental ulcer models in rats. **Adv Pharmacol Toxicol,** v. 17, p. 37-44, 2016.

RIVERA, D. E. O.; CAMPO, Y. C.; CASTRO, J. P.; CARO, D.; FRANCO, L. A Antibacterial activity of *Physalis angulata* L., *Merremia umbellata* L., and *Cryptostegia grandiflora* Roxb. Ex R.Br.–medicinal plants of the Colombian Northern Coast. **Orient Pharm Exp Med**, v. 15, p. 95-102. 2015.

ROBLEDO-TORRES, V.; RAMÍREZ-GODINA, F. FOROUGHBAKHCH-POURNAVAB, R.; BENAVIDES-MENDOZA, A. HERNÁNDEZ-GUZMÁN, G.; REYES-VALDÉS, H. M. Development of tomatillo (*Physalis ixocarpa* Brot.) autotetraploids and their chromosome and phenotypic characterization. **Breed Sci**, v. 61, p. 288-293, 2011.

SÆBØ, A.; KREKLING, T.; APPELGREN, M. Light quality affects photosynthesis and leaf anatomy of birch plantlets in vitro. **Plant Cell Tissue Organ Cult**, v. 41, p. 177-185,1995.

SABAGHNIA, Z.; MATLOOBI, M.; MOTALLEBI-AZAR, A. Response of leaf number and plant height of polka dot plant (*Hypoestes phyllostachya*) under various indoor lights. **Agricult Forest**, v. 62, p. 287-294, 2016.

SANG-NGERN, M.; YOUN, U. J.; PARK, E. J.; KONDRATYUK, T. P.; SIMMONS, C. J.; WALL, M. M.; RUF, M.; LORCH, S. E.; LEONG, E.; PEZZUTO, J. M. Withanolides derived from *Physalis peruviana* (Poha) with potential anti-inflammatory activity. **Bioorg Med Chem Lett,** v. 26, p. 2755-2759, 2016.

SILVA, B. J. M.; SILVA, R. R. P.; RODRIGUES, A. P. D.; FARIAS, L. H. S.; NASCIMENTO, J. L. M.; SILVA, E. O. *Physalis angulata* induces death of promastigotes and amastigotes of *Leishmania amazonenses* via the generation of reactive oxygen species. **Micron**, v. 82, p. 25-32, 2016.

SILVA, B. O.; AMARAL, A. C. F.; FERREIRA, J. L. P.; SANTIAGO, L. J. M.; LOURO, R. P. Micropropagation and *in vitro* production of secondary metabolites of *Croton floribundus* Spreng. *In Vitro* Cell Dev Biol, **Plant**, v. 49, p. 366-372, 2013.

TERASHIMA, I.; HANBA, Y. T.; THOLEN, D.; NIINEMETS, Ü (Leaf functional anatomy in relation to photosynthesis. **Plant Physiol**, v. 155, p. 108-116, 2011.

THEPSITHAR, C.; THONGPUKDEE, A Comparative micro-morphology, anatomy and architecture of leaf of *Physalis*. **World Acad Science Eng Technol**, v. 7, p. 806-809, 2013.

TRUPKIN, S. A.; LEGRIS, M.; BUCHOVSKY, A. S.; TOLAVA-RIVERO, M. B.; CASAL, J. J. Phytochrome B nuclear bodies respond to the low red to far-red ratio and to the reduced irradiance of canopy shade in *Arabidopsis*. **Plant Physiol**, v. 165, p. 1698-1708, 2014.

UZUN, S. The quantitative effects of temperature and light on the number of leaves preceding the first fruiting inflorescence on the stem of tomato (*Lycopersicon esculentum*, Mill) and aubergine (*Solanum melongena* L.). **Scientia Hortic**, v. 109, p. 142-146, 2006.

WANG, L.; XIN, M.; QIN, Z.; LIU, H. Functional analysis of an iaaM gene in parthenocarpic fruit development in transgenic *Physalis pubescens* L. plants. **Plant Cell Tissue Organ Cult**, v. 107, p. 333-340, 2011.

WANG, X. Y.; XU, X. M.; CUI, J. The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of *Cucumis sativus* grown under weak light. **Photosynthetica**, v. 53, p. 213-222, 2015.

WANG, Y.; FOLTA, K. M. Contributions of green light to plant growth and development. **Am J Bot**, v. 100, p. 70-78, 2013.

WU, C. F.; KARIOTI, A.; ROHR, D.; BILIA, A. R.; EFFERTH, T (Production of rosmarinic acid and salvianolic acid B from callus culture of *Salvia miltiorrhiza* with cytotoxicity towards acute lymphoblastic leukemia cells. **Food Chem,** v. 201, p. 292-297, 2016.

XU, Y. M.; BUNTING, D. P.; LIU, M. X.; BANDARANAYAKE, H. A.; GUNATILAKA, L. A. A. 17β-Hydroxy-18-acetoxy with anolides from aeroponically grown *Physalis crassifolia* and their potent and selective cytotoxicity for prostate cancer cells. **J Nat Prod**, v. 79, p. 821-830, 2016.

YEH, N.; CHUNG, J. P. High-brightness LEDs-Energy efficient lighting sources and their potential in door plant cultivation. **Renew Sustainable Energy Rev**, v. 13, p. 1-6, 2009.

YOKAWA, K.; KAGENISHI, T.; BALUŠKA, F Root photomorphogenesis in laboratory-maintained *Arabidopsis* seedlings. **Tech Appl**, v. 18, p. 117-119, 2013.

YÜCESAN, B. B.; MOHAMMED, A.; ARSLAN, M.; GÜREL, E. Clonal propagation and synthetic seed production from nodal segments of cape gooseberry (*Physalis peruviana* L.), a tropical fruit plant. **Turk J Agric For**, v. 39, p. 797-806, 2015.

Article 2 - Lighting and sucrose in photoautotrophic and photomixotrophic micropropagation of *Physalis angulata*

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ABSTRACT

Biotechnological interest in *Physalis* has increased in recent decades. However, there are still few micropropagation researches. An investigation was conducted to evaluate photoautotrophic and photomixotrophic micropropagation of *Physalis angulate*, gas exchange, seven types of lighting and five concentrations of sucrose. Lighting treatment consisted of yellow, blue, white, red, green, red + blue LEDs and natural light filtered by screen. The concentrations of sucrose were 0, 7.5, 15, 22.5 and 30. Phytotechnical, anatomical and photopigmentation characteristics were evaluated. These consisted of segment, stem and root length, leaf number and area, chlorophyll a, b and, carotenoid content, adaxial and abaxial epidermis, palisade and spongy parenchyma. Data were compared by Scott-Knott's mean test and principal component analysis in software R. Within lighting treatment, only screen, screen-filtered natural illumination, obtained maximum evaluation in all variables. Within sucrose treatment, 15 sucrose obtained the highest number of means with maximum evaluation. The results showed that screen made viable photoautotrophic micropropagation of *P. angulata*. Furthermore, photomixotrophic micropropagation with 15 g / L of sucrose provided better results.

Key words: Applied botany. Ecological anatomy. Small fruit crops. Vegetal tissue culture. Photopigments.

1 INTRODUCTION

The first works on plant tissue culture used the heterotrophic system in which a culture medium has the presence of a carbon source and the container has plastic cap to prevent drying and contamination of culture medium (MURASHIGE; SKOOG, 1962). Subsequently, emerged some works using gas exchange with external medium system and no sucrose on culture medium, classified as photoautotrophic micropropagation (KOZAI, 1991). In addition to normalizing gases concentration inside the tube, the photoautotrophic micropropagation decreases energy expenditure and culture medium contamination (XIAO et al., 2011).

Photoautotrophic micropropagation helps the acclimatization phase, improving the anatomical and physiological characteristics that allow the survival and development of the seedlings (WU; LI, 2013; MARTINS et al., 2015). The increase of transpiration, provided by the photoautotrophic micropropagation, avoids the explant hyperhydricity (KOZAI et al., 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 cuticle and formation of nonfunctional stomata, causing higher explants mortality (SALDANHA et al., 2012; CHANDRA et al., 2010).

Despite above advantages, photoautotrophic micropropagation difficulty of implantation is the higher cost of mechanized systems and 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 et al., 2013). Commercial membranes such as MilliSeal®, successfully used in orchid micropropagation (SILVA, 2013), are currently on the market. However, other researchers have successfully evaluated the use of low-cost, handcrafted porous lids such as cotton swabs (SALDANHA et al., 2012).

Sucrose also interferes in efficiency of micropropagation with gas exchange. In a total absence of sucrose in culture medium, we have the photoautotrophic micropropagation, however, when we add sucrose the designation 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 authors observed better development of those 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 as high intensity of light and cold in seedlings (TOGNETTI et al., 2013; BADR et al., 2011).

Another challenge of photoautotrophic micropropagation is to establish a better lighting protocol. Plants need light to regulate their development, physiology and morphogenesis (HUCHÉ-THÉLIER et al., 2016). In growth rooms, lighting has mainly fluorescent lamps or light emitting diodes (LEDs) (BIAN et al., 2014). Between these two options, LEDs have advantages such as high efficiency in light generation process, low heat production and availability at different well-defined wavelengths (YEH; CHUNG, 2009). The photoautotrophic micropropagation, that uses natural light, is a little explored technique in plant tissue culture, and has great potential for drastic reduction of seedling production costs (ERIG, SCHUCH, 2005).

The genus *Physalis* (Solanaceae) is a taxonomic group easily recognized by a peculiar fruiting morphology, which is characterized by the presence of an additive and inflated fruiting chalice enveloping the fruit (SOARES et al., 2009). The number of *Physalis* species is between 75 and 90. The center of diversity of this group is in Mexico, where two thirds of the species are endemic (SOARES et al., 2009). In South America, there are approximately 12 species. (SOARES et al., 2009). The plants of this genus are widely used in the world cuisine, like in bittersweet salads, jellies, pies, cakes, juices, seasonings and sauces (PUENTE et al., 2011). However, there are other applications besides feeding. A number of studies have been carried out at the biotechnological level with the culture of *Physalis* (BERGIER et al., 2012), and it includes micropropagation (KHAN; BAKHT, et al., 2009).

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* researches 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.

2 MATERIAL AND METHODS

2.1 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). We sealed tubes 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. Subcultures occurred every 30 days, in a total of 3 subcultures.

2.2 Experimental procedures

We inoculated nodal segments (1cm) 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}. We used handmade cotton lids, which allowed the uniform passage of air. The lighting treatments consisted of screen (natural light filtered by a Polysack's Cromatinet® black photoconverter mesh with 50% shading), and six different tubular LED lamps, TEC-LAMP® (TECNAL®) model, at the following wavelengths (660 nm and 75 μmol m^{-2}, s^{-1}), blue + red (660 nm + 450 μm) (525 nm and 74 μmol, m^{-2}, s^{-1}), and yellow (525 nm and 76 μmol m^{-2}, s^{-1}), white (Cool White 7,000 and 75 μmol m- (590 nm and 74 μmol, m^{-2}, s^{-1}.

After 30 days of in vitro culture, analyzes wer performed:

2.2.1 Gas exchange analysis

We measured concentrations of O_2 and CO_2 inside the tubes with the use of a PBI Dansensor® respirometer. Calibration occurred on external atmosphere, 21.1% O_2 and 0.1% CO_2. We inserted an apparatus needle into each tube by drilling a cotton cap.

2.2.2 Phytotechnical characteristics

We measured the length of nodal segments, stem and root. We evaluated total leaf area using a desktop scanner and ImageJ software version 1.49 p.

2.2.3 Content of photosynthetic pigments

Chlorophyll content assay was based on methodology without leaf maceration according to the methodology of Macedo et al. (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. 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}).

2.2.4 Leaf anatomy

We placed median region rectangular fragments of second leaf placed in F.A.A.70% (JOHANSEN, 1940) for a period of 72 h at 10 ° C. Then the fragments undergone

dehydration in ethanoic series of 70%, 80%, 90% and 100% for 2h, at 10 ° C each. The first step of resin infiltration in the samples occurred on a 100% ethanol solution and 1: 1 liquid methacrylate resin, per 24 hours. The second step of the infiltration occurred on pure and activated resin for 72 h at 10 ° C. Confection of blocks containing material occurred with the use of activated resin solution and hardener in an oven at 37 ° C until solidification. Cross sections of 10 μm 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. 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). 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).

3 RESULTS

Comparing the variables within lighting types, we observed that only the screen treatment, screened by mesh, obtained maximum evaluation in all means (TABLE 1). In addition, we observed that micropropagated seedlings without sucrose only grew in combination with screen treatment (FIGURE 1). In the zero sucrose treatment, only the combination with screen provided material for making blades (FIGURE 2).

Table 1 - 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). 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.

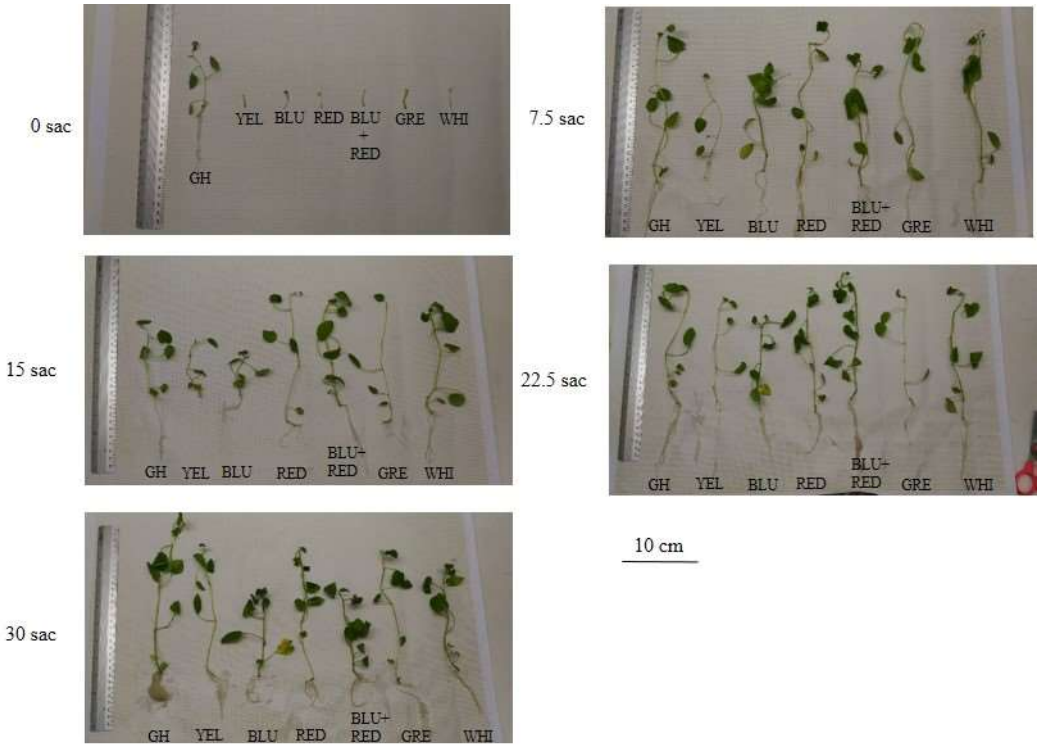
Sucrose	O_2		CO_2		Segment		Stem		Root	
0	21,10	b	0,10	a	1,50	d	5,53	b	9,47	a
7,5	21,10	b	0,10	a	2,60	c	9,70	b	8,53	a
15	21,17	b	0,10	a	4,20	b	20,50	a	9,67	a
22,5	21,40	a	0,07	a	3,97	b	21,30	a	10,10	a
30	21,40	a	0,10	a	5,47	a	20,43	a	10,50	a
CV (%)	0,49		55,33		5,30		22,07		6,93	

Sucrose	Leaf Number		Leaf Area		Chlorophyll a		Chlorophyll b		Total Chlorophyll	
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		Adaxial		Palisade		Spongy	
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	34,84	b
CV (%)	15,93		24,88		5,32		7,73		9,29	

Sucrose	Abaxial		Leaf Thickness	
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	

Figure 1 - Plants of *P. angulata* micropropagated with gas exchange and treated in factorial scheme 7 (types of illumination) x 5 (concentrations of sugar). 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.



Comparing the levels of sucrose, we observed that the sucrose treatment obtained the highest number of maximum evaluation averages (TABLE 2). The treatment 15 was the only one that obtained averages with maximum evaluation in the variables of photopigments. In addition, the sucrose treatment obtained a maximum score in the two main anatomical variables, palisade and spongy parenchyma.

Table 2 - Mean test of analyzed variables in *P. angulata* micropropagated with gas exchange, treated with five different concentrations of sucrose, focusing only on Screen source of variation. 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. (to be continued)

Lighting	O_2		CO_2		Segment		Stem		Root	
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	

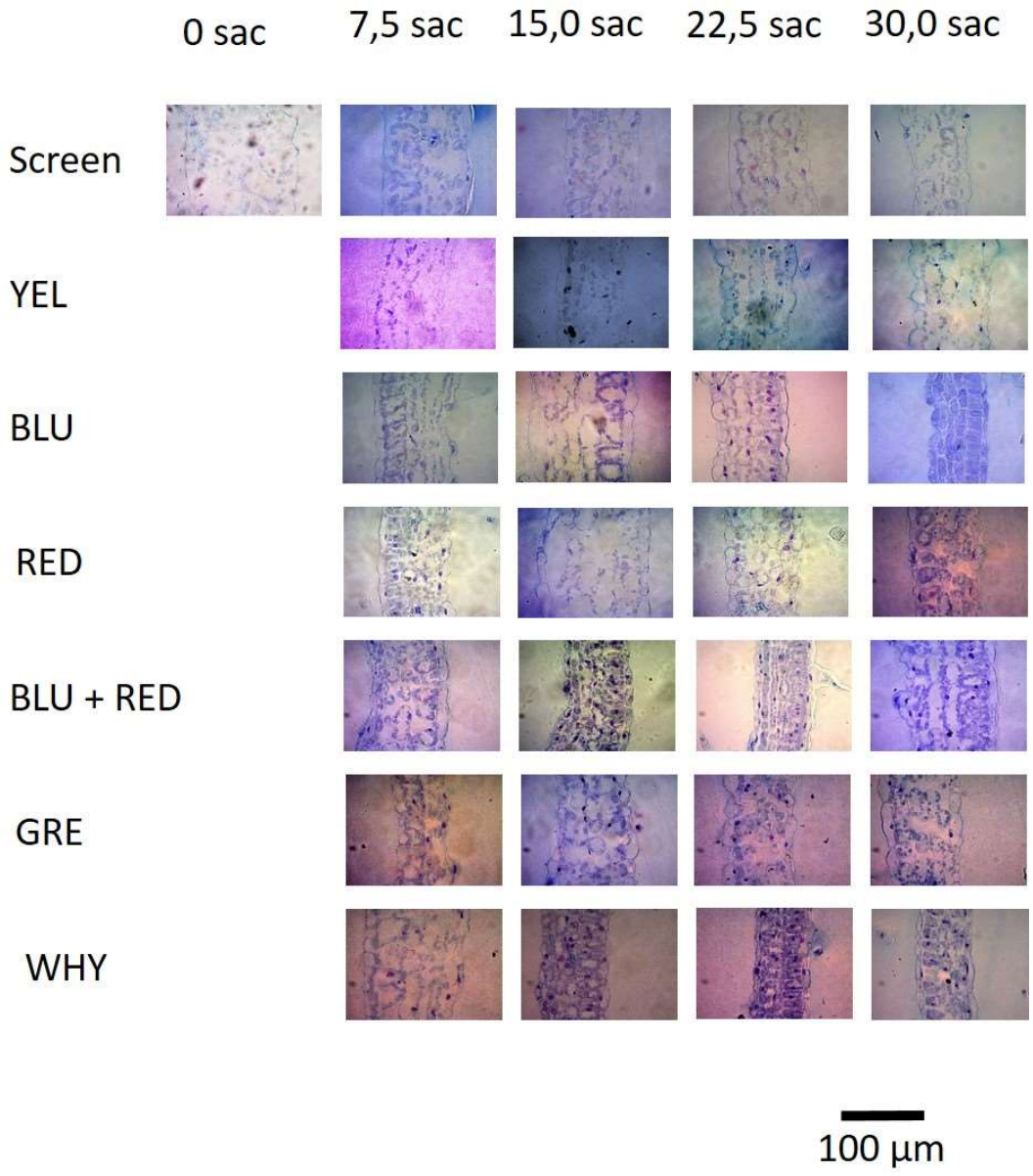
Lighting	Leaf Number		Leaf Area		Chlorophyll a		Chlorophyll b		Total Chlorophyll	
Blue	5,47	a	106.006,93	a	0,43	b	0,30	b	0,73	b
Blue + Red	6,20	a	117.143,47	a	0,42	b	0,37	b	0,79	b
Green	3,93	b	82.393,47	b	0,33	b	0,40	b	0,73	b
Red	4,73	b	92.489,13	b	0,14	b	0,21	b	0,35	b
Screen	6,07	a	123.721,33	a	1,39	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		Adaxial		Palisade		Spongy	
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	

Table 2 - Mean test of analyzed variables in *P. angulata* micropropagated with gas exchange, treated with five different concentrations of sucrose, focusing only on Screen source of variation. 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. (conclusion)

Lighting	Abaxial		Leaf Thickness	
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	

Figure 2 - Leaves cross sections of *P. angulata* that were 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 principal component analysis, the results showed that leaf number, chlorophyll a / b, palisade, spongy, abaxial and leaf thickness were strongly correlated (TABLE 3). In the

analysis of multiple factors, the results showed that the screen treatment had a low correlation with the others, which in turn had a high correlation with each other (TABLE 4).

Table 3 - Correlation of the main components with the original variables for the means of analyzed variables in micropropagated *P. angulata* with gas exchange, treated in factorial scheme 7 (types of illumination) x 5 (concentrations of sucrose). UFLA, Lavras, MG, Brazil, 2017.

Comp.	O_2	CO_2	Segment	Stem	Root	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	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Chlorophyll a/b	Total Carotenoids
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	Palisade	Spongy	Abaxial	Leaf Thickness
1	0,773	0,925	0,895	0,843	0,923
2	-0,277	0,025	-0,093	-0,297	-0,129

Table 4 - Inertial values computed in the first three axes for each group of observations in micropropagated *P. angulata* with gas exchange, treated in factorial scheme 7 (types of illumination) x 5 (concentrations of sucrose). UFLA, Lavras, MG, Brazil, 2017.

Componentes	Grupos de Lighting						
	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

4 DISCUSSION

In the zero sucrose treatments, the results showed that luminous intensity of the LEDs was not enough to stimulate the photosynthesis to the point of promoting the development of the axillary buds of the nodal segments (FIGURE 1). A totally photoautotrophic micropropagation occurred only under natural light filtered by the mesh with 50% shading, screen treatment. In presence of sucrose in culture medium, seedlings developed normally. A low photon flux rate of lamps used in the growth chamber is a challenge known to researchers in tissue culture area (KOZAI; NGUYEN, 2003). Electric energy expenditure is a great

obstacle for increasing quantity or power of the lamps (ERIG; SCHUCH, 2005). Multiple factor analysis clearly showed that there was a difference between the screen treatment and other treatments with artificial light (TABLE 4). There is a great interest in the use of natural light in plant tissue culture (ERIG, SCHUCH, 2005), so the phytotechnical variables of *Physalis* under this condition were analyzed separately (TABLE 2). Results showed that concentration 15 g / L sucrose obtained better results in relation to other treatments, mainly in photopigments (TABLE 2). This result indicates that the sucrose concentration 15 g L-1 provided balanced carbon amount, which allowed promoting development of the axillary bud, without inhibiting the formation of anatomical structures, and it promoted the expression of photopigments. This result reduces in half the sucrose concentration of the traditional MS medium (MURASHIGE; SKOOG, 1962). Tomato grows better under photoautotrophic conditions (KUBOTA; TADOKORO, 1999), indicating a difference in behavior within the same botanical family of *Physalis*.

In all treatments, a layer of adaxial epidermis, a palisade parenchyma layer perpendicular to the epidermis, three to four layers of spongy parenchyma with irregularly shaped cells, bicolateral bicuspid vascular bundle and a layer of abaxial epidermis (Figure 2). Therefore, the treatments provided tissue layout and amount of cell layers similar to that observed in field-grown Physalis (THEPSITHAR; THONGPUKDEE, 2013). It was observed that the zero sucrose treatment had a greater thickness of palisade parenchyma, spongy and foliar thickness in relation to the other treatments (TABLE 1). 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 2). 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 tissue inhibition is a reason why photosynthetic assimilation of CO_2 decrease in the presence of sucrose in medium (COUTO et al., 2014). These results also indicate that stress is present during the formation of seedlings due to excess sucrose (TOGNETTI et al., 2013; BADR et al., 2011).

By main component analysis, the results showed a strong correlation between important variables for light capture and photosynthesis: leaf number, chlorophyll a / b, palisade, spongy, abaxial and leaf thickness (TABLE 3). 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 et al., 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), with differences in thickness of this tissue 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).

Article 3 - Colchicine in the induction of polyploids in *Physalis alkekengi*

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ABSTRACT

Physalis alkekengi is an ornamental plant, but there is a growing interest in its medicinal application due to its anti-inflammatory, bactericidal, antitumor and fungicidal properties. Polyploidization can be an important tool in the genetic improvement of this species. The objective of this study was to obtain in vitro and evaluate the phytotechnical characteristics of tetraploides *P. alkekengi*. For this goal, we inoculated nodal segments of *P. alkekengi* var. Franchettii into petri dishes containing 100 ml of MS medium supplemented with colchicine at concentrations 0; 0.04; 0.08; 0.12; and 0.16% and kept in the dark for 24 and 48h. After the respective treatment periods with colchicine the segments were inoculated into test tubes. We identified tetraploids by flow cytometry and classical cytogenetics. In vitro seedlings were measured in: root length, root length, nodal segment length, leaflet number and total leaf area. In the acclimatization phase, area of the second leaf and total leaf, petiole radius, stem length, weight of the fruit with the calyx, without the calyx, the fruit diameter, the number of seeds and brix of the pulp were evaluated. We also estimated Chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, total chlorophyll / total carotenoid ratio and chlorophyll a / b ratio. The treatment that most produced tetraploid seedlings was with 0.08% colchicine, for 24h. No significant difference was observed in 7 (seven) variables, these being all variables of photopigments, stem diameter (steam) and brix. In general, diploid (2x) plants were better in 9 (nine) while tetraploid seedlings were better in 6 (six) of the phytotechnical variables. We concluded that the MS medium supplemented with 0.08% colchicine for 24 h allowed to obtain *P. alkekengi* tetraploides with better phytotechnical qualities.

Key words: plant tissue culture, plant biotechnology, plant breeding, genetic improvement, applied botany

1 INTRODUCTION

Physalis alkekengi is a plant of *Solanaceae* family easily identified by an additional chalice of bright orange color covering the small fruit and by flowers with white and lobed corolla (WANG et al., 2014). The main varieties are Alkekengi and Franchetii, the latter with larger fruits and with distinct spots at the base (WANG et al., 2014). The main application of the Franchetii variety is ornamental (POPA-MITROI et al., 2012), but there is a growing interest in its medicinal application due to its anti-inflammatory (HONG et al., 2015) bactericidal properties (ZHANG et al., 2016), antitumor (LI et al., 2014) and fungicides (TORABZADEH; PANAHI, 2013).

Polyploidization in plants is a phenomenon that occurs naturally and is important for sympatric speciation (ARVANITIS et al., 2010). Approximately 70% of spermatophytes are tetraploids (ZHANG et al., 2012). Biotechnologically, there are several protocols for polyploidization, and in vitro realization is an excellent way to obtain clones of the tetraploid and to evaluate them always maintaining a copy (OLIVEIRA et al., 2013). Its occurrence can result in characteristics beneficial to the plant as drought tolerance (MANZANEDA et al., 2012). In the pharmacological area, polyploidization may increase the production of substances of interest by plants (MAJDI et al., 2010). There is growing interest in identifying and developing new genotypes of *Physalis* with different objectives. Researchers know that in *P. peruviana*, the degree of ploidy interferes in the resistance to the phytopathogenic fungus of the genus *Fusarium* (LIBERATO et al., 2014; OSORIO-GUARÍN et al., 2016). In *P. ixocarpa*, there are studies comparing the fruit quality of diploid and tetraploid plants (RAMÍREZ-GODINA et al., 2013).

In view of the above, we aim to obtain tetraploids of *P. alkekengi* var. Franchetii and evaluates them for in vitro and ex vitro plant breeding characteristics.

2 MATERIAL AND METHODS

2.1 Obtaining biological material

Seeds of *P. alkekengi* var. Franchetii underwent 70% alcohol asepsis for 10 min, 50% sodium hypochlorite for 20 min and four washes with autoclaved distilled water. The seeds were germinated in vitro in MS medium (MURASHIGE; SKOOG, 1962) without growth regulators, at an average temperature of 25 ± 2 ° C, with a translucent plastic lid, in a growth room with photoperiod 16h, with white fluorescent lamps and 35 μmol m-2 s-1 of irradiance. Seedlings underwent three subcultures under the same conditions as above.

The adult plants were obtained by acclimatization in a 25 L pot containing Plantmax substrate, in a greenhouse with black photoconverter mesh Cromatinet ® Polysack with 50% shading. The spacing between the vessels was 20 cm. The experiment was carried out at the Moacir Pasqual Tissue Culture Laboratory, at the Federal University of Lavras, in the city of Lavras, in the state of Minas Gerais, Brazil. The region is located at latitude 21 ° 14 '43 south and longitude 44 ° 59' 59 west, being at an altitude of 918 meters (DANTAS et al., 2007). Ex vitro growth occurred in June, July and August 2017, in which historical temperatures range from 12 to 19 ° C (ACCUWEATHEr, 2017).

2.2 **Experimental treatment:** induction of polyploidy in vitro

After the third subculture, seedlings were separated for induction of polyploids. Nodal segments of *P. alkekengi* var. Franchettii were inoculated into petri dishes containing 100 ml of MS medium (MURASHIGE; SKOOG, 1962) supplemented with filtered and sterilized colchicine at 0, 0.04; 0.08; 0.12; and 0.16% and kept in the dark for 24 and 48h. Thus, 10 treatments (5 concentrations x 2 times in colchicine) were performed. For each treatment 50 nodal segments of *P. alkekengi* were used, totaling 500 segments. After the respective treatment periods with colchicine, the explants underwent triple washing in distilled and autoclaved water. The segments were then inoculated into test tubes, under the same micropropagation conditions as mentioned above.

2.3 Flow cytometry analyzes

After 30 days of in vitro growth, a number of possible mixoploids, diploids and tetraploids were observed by estimating the amount of DNA in flow cytometry. For this, approximately 50 to 60 mg of young *P. alkekengi* leaflets were used per treatment, along with corresponding sample of internal reference standard, tomato (*Solanum lycopersicum* cv. Stupické). The material was ground with a scalpel in a Petri dish containing 1 mL of ice-cold Marie buffer to release the nuclei (DOLEZEL; BINAROVA; LUCRETTI, 1989). The core suspension was aspirated and subsequently filtered through 50 μm mesh filters. The cell suspension was kept in a container with crushed ice so that no deterioration of the material occurred. The nuclei were then stained by the addition of 25 μl of propidium iodide in each sample. Five thousand nuclei were analyzed for each sample, with three replicates. The analysis was performed on the FACSCalibur four-color cytometer (Becton Dickinson) and the histograms obtained and analyzed in the Cell Quest software. The nuclear (pg) DNA content of the plants was estimated by comparison with the position relative to the G1 peak of the internal reference standard.

2.4 Cytogenetic analysis

Root tips 1 cm long were pre-treated with 0.002 M 8-hydroxyquinoline (8-HQ) for 24 h at 4 ° C (Guerrero, Souza, 2002). The roots were fixed in Carnoy 3: 1 (ethanol / acetic acid v / v) for 24 hours and then stored at -20 ° C. The slides were prepared according to the crushing technique and stained with 2% gummer. For the capture of the images a microscope of clear field equipped with microcamera was used.

2.5 Phytotechnical analyzes

For in vitro seedlings, the length of the root (cm), the length of the nodal segment (cm), and the number of leaflets were measured with the aid of a pachymeter. It was also evaluated the total leaf area (cm^2) with the use of desktop scanner and a java image processing and analysis software ImageJ version 1.49p. In the adult plants, acclimatization

phase, total leaf area (cm^2) and second leaf (cm^2) were evaluated. The petiole radius of the second leaf (mm) and the stem length (cm) were also measured. In the reproductive part, the weight of the fruit was measured with the calyx (g) and without the calyx (g), fruit diameter (mm), number of seeds and brix of pulp (%).

2.6 Biochemical analyzes

The test of chlorophyll content was based on methodology without leaf maceration (SCOPEL et al., 2011; MACEDO et al., 2013). Leaflets in a total of 0.5 g were deposited in capped test tubes containing 10 mL of 80% (v / v) acetone. The tubes containing the material were incubated for 24 h at 5 ° C, protected from light. At the end of this period, the resulting solution was transferred to cuvettes for spectrophotometer analysis. The reference sample consisted of 80% (v / v) acetone solution. Absorbance readings were performed at wavelengths 645, 652, 663 and 470 nm. Chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, total chlorophyll ratio / total carotenoids, and chlorophyll a / b ratio were calculated with the aid of the estimators: 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. The results were expressed in milligram of pigment per gram of fresh matter weight ($mg.g^{-1}$).

2.7 Statistical analysis

The experiment was installed in a completely randomized design with 5 (five) replicates. 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; CAVALCANTI; NOGUEIRA, 2013).

3 RESULTS

Four types of seedlings were obtained: non-survivors, mixoploids (2x + 4x), diploids (2x) and tetraploids (4x) (FIGURE 1, FIGURE 2). Table 1 shows that the treatment that most produced tetraploid seedlings was with 0.08% colchicine, for 24h. Non-surviving seedlings showed necrosis with the treatments. Therefore, it was confirmed that they were not only with dormant axillary buds (FIGURE 1). The mixoploid seedlings presented irregular development, deformations and necrotic tissues that were easily visible in the aerial part (FIGURE 1). The diploid and tetraploid seedlings presented healthy development, being the tetraploids more robust and with larger leaves (FIGURE 1). Survival and ploidy estimated by cytometry were evaluated (TABLE 1). The survival of the explants was 100% in the treatments with 0% conchicine and decreased as the concentration of the antimitotic was increased. In addition, it was observed that exposure time of 48 h resulted in higher mortality of explants than 24 h for all treatments with colchicine (TABLE 1). The DNA content estimated by flow cytometry was practically double in the tetraploid seedlings in relation to the diploids (TABLE 1).

Figure 1 - Seedlings of *P. alkekengi* var. franchettii treated with colchicine. Non-surviving (ns), myxoploid (2x + 4x), diploid (2x) and tetraploid (4x) seedlings were obtained. UFLA, Lavras, MG, Brazil, 2017.

Figure 2 - Histograms of flow cytometry for *P. alkekengi* var. franchettii mixoploides (2x + 4x), diploids (2x) and tetraploids (4x) and standard (tomato). UFLA, Lavras, MG, Brazil, 2017.

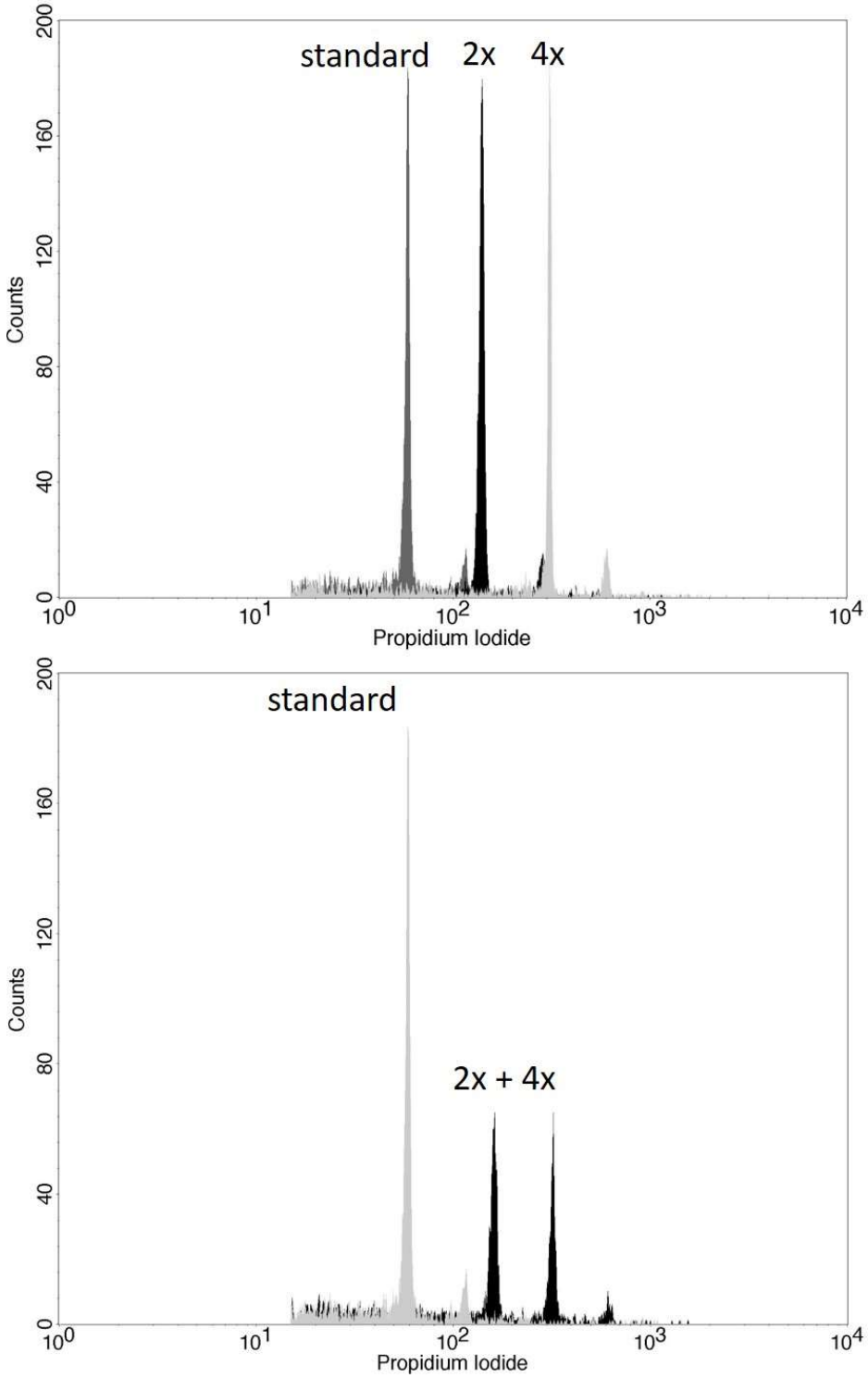


Table 1 - Percentage of survival, number of seedlings for each ploidy obtained in *P. alkekengi* and DNA content for diploids and tetraploids. UFLA, Lavras, MG, Brazil, 2017. Legend: 2x = diploid, 4x = tetraploid, 2X + 4X = myxoploid. Means followed by the same letter do not differ by 5% probability.

Colchicine (%)	Time (h)	Survival (%)	2x	4x	2x+4x
0	24	100	50	0	0
	48	100	50	0	0
0,04	24	72	24	3	9
	48	44	18	2	2
0,08	24	66	13	8	12
	48	18	3	4	2
0,12	24	32	4	5	7
	48	12	0	5	1
0,16	24	12	4	5	7
	48	6	1	0	2
DNA contente (pg)			4,82 b	10,46 a	-
CV (%)			3,1	20,42	-

By the mean test (TABLE 2), no significant difference was observed in 7 (seven) variables, all of them being photopigments, stem diameter (steam) and brix. In general, diploid (2x) plants were better in 9 (nine) while tetraploid seedlings were better in 6 (six) of the phytotechnical variables. However, comparing the variables analyzed in vitro, 4x presented greater leaflet number and total leaflet area, while 2x was better at shoot and radicle length. Regarding the adult plant (FIGURE 3), 4x took longer to bloom and had longer root length. The adult diploid plants presented heavier fruits, both with the capsule and without the capsule, larger fruits and with greater number of seeds (FIGURE 4).

Table 2 - Scott-Knott test for the variables analyzed in adult explants and diploid and tetraploid plants of *P. alkekengi* var. franchettii treated with different concentrations of colchicine and exposure times. Means followed by the same letter do not differ by 5% probability. UFLA, Lavras, MG, Brazil, 2017.

Ploidy	Leaflet Number		Shoot (cm)		Radicle (cm)		Segment (cm)		Total Leaflet Area (cm^2)	
2X	8,00	b	17,56	a	6,22	a	3,84	a	5,86	b
4X	11,00	a	12,76	b	4,66	b	1,76	b	10,60	a
CV (%)	11,77		6,94		11,59		7,82		11,38	

Ploidy	Chl a (mg/g)		Chl b (mg/g)		Total Chl (mg/g)		Chl a/b		Carotenoids (mg/g)	
2X	0,00176	a	0,00205	a	1,13	a	1,49	a	0,05	a
4X	0,00133	a	0,00068	a	1,80	a	2,14	a	0,08	a
CV (%)	128,49		200,17		48,07		39,98		52,09	

Ploidy	Total Leaf Area (cm^2)		Leaf Area (cm^2)		Petiole Ray (mm)		Leaf Number		Stem (cm)	
2X	772,44	a	27,84	b	2,60	b	40,00	a	15,40	b
4X	304,64	b	58,96	a	3,40	a	8,20	b	21,20	a
CV (%)	0,89		7,88		18,26		5,25		3,74	

Ploidy	Stem Ray (mm)		First Flowering (days)		Fruit with Chalice (g)		Fruit without Chalice (g)		Fruit Diameter (mm)	
2X	5,00	a	32,60	b	2,28	a	1,78	a	14,20	a
4X	5,12	a	61,60	a	1,11	b	0,78	b	10,20	b
CV (%)	4,98		7,22		32,31		36,27		10,69	

Ploidy	Brix (%)		Seeds Number	
2X	14,94	a	187,80	a
4X	13,10	a	29,40	b
CV (%)	9,32		8,43	

Figure 3 - Adult plants of *P. alkekengi var*. franchettii diploid (2x) and tetraploid (4x). UFLA, Lavras, MG, Brazil, 2017.

Figure 4 - Fruits with calisse and without calisse of *P. alkekengi* var. franchettii diploid (2x) and tetraploid (4x). UFLA, Lavras, MG, Brazil, 2017.

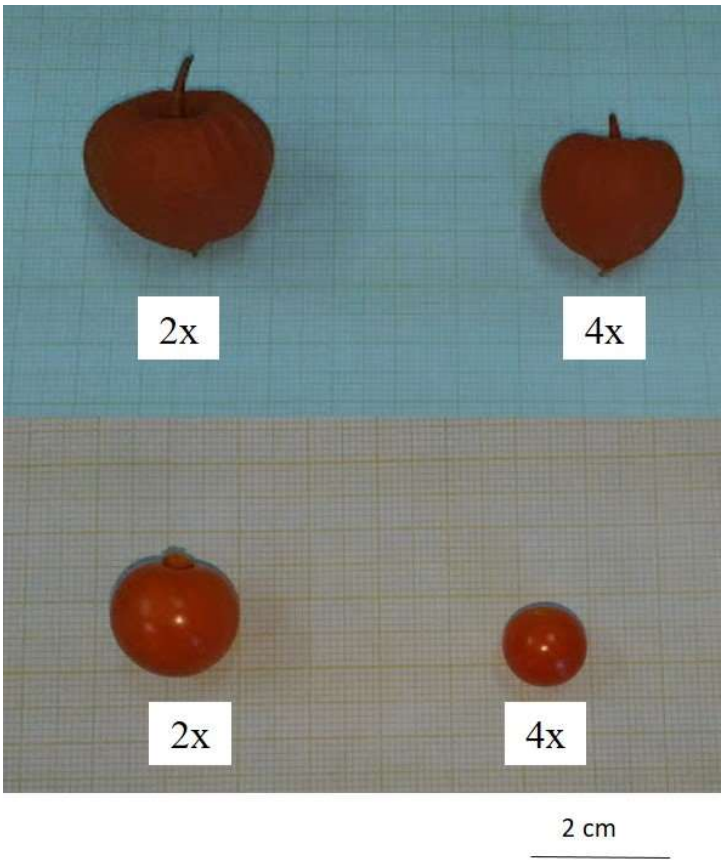
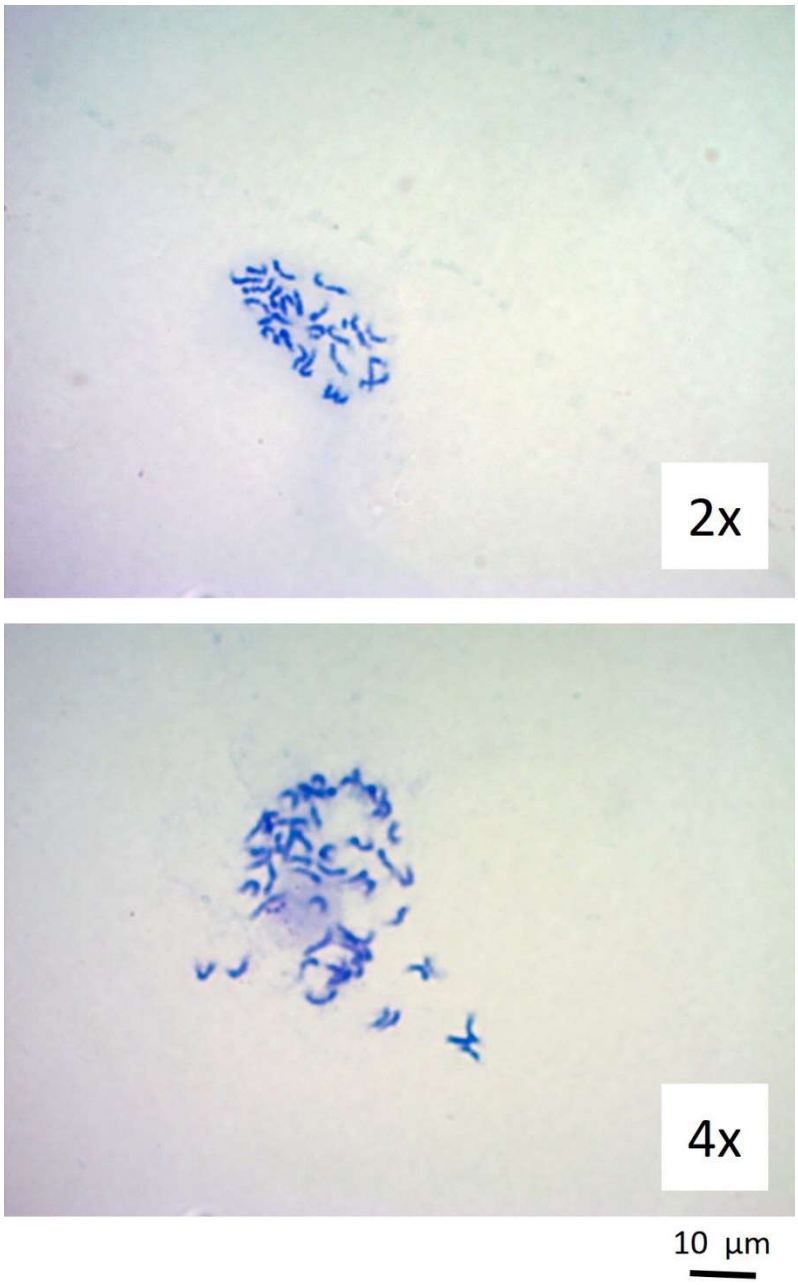


Figure 5 - Micrograph of cryptogenetic analysis for *P. alkekengi* var. franchettii diploides (2x) and tetraploids (4x), respectively with 24 and 48 chromosomes. UFLA, Lavras, MG, Brazil, 2017.



4 DISCUSSION

The best treatment to obtain tetraploids was with 0.08% colchicine in the MS medium for 24 h, in which 8 tetraploids were observed (TABLE 1, FIGURE 1). With this result, it was possible to halve the time of obtaining tetraploids of *P. alkekengi* in comparison to Nakamura et al. (2007). The appearance of mixoploids was also observed (Table 1, Figure 1). To avoid the appearance of this type of mutant, it would be necessary to use cell suspension for treatment with colchicine (ACANDA et al., 2015). As expected in relation to DNA content, tetraploid seedlings presented twice as much genetic material as diploids (TABLE 1, FIGURE 2). The flow cytometry plot shows the G2 peak of the diploid coinciding with the G1 peak of the tetraploid (FIGURE 2), which indicated that the chromosome duplication was successful. From Figure 5, we observed that the duplication was confirmed cytogenetically. As expected, *P. alkekengi* diploides seedlings have 24 chromosomes (BADR et al., 1997) and tetraploids 48 (FIGURE 5).

As observed by Nakamura et al. (2007), the *P. alkekengi* diploid plants of this experiment also showed higher fruits, higher number of seeds, higher number of leaves (Table 2). However, tetraploid results have shown that these tend to invest more energy in leaf size (Table 2). One explanation may be the delay to early flowering and fruiting in relation to diploid plants (TABLE 2). However, molecular and ecological studies must be carried out to discover the advantages of this variety of *P. alkekengi* tetraploid with such characteristics. Tetraploid seedlings may produce more medicinal substances than diploids (JESUS-GONZALEZ; WEATHERS, 2003). In other cases, tetraploids may be more tolerant to abiotic stresses (SALEH et al., 2008; LIU et al., 2011). Negative changes may also occur. For example, there are tetraploids that develop invasive growth relative to the diploid variety (SCHLAEPFER et al., 2010). All these analyzes can be carried out in the future, since the tetraploid obtained in this work is well established, producing fruits and seeds. In any case, the main application of *P. alkekengi* is ornamental, an area that also values flowers and fruits of smaller size.

By our results, allied to the results of Nakamura et al. (2007), it was observed that the tetraploids of *P. alkekengi* present many anatomical characteristics of smaller size than that observed in the diploid plants (TABLE 2, FIGURE 3, FIGURE 4). These results are the

opposite of what was expected in tetraploid plants, which usually present an increase of the organs by the "gigas" effect. The explanations for the results cited above would be the reduction of the number of cell divisions and lower growth rate (SATTLER et al., 2016).

5 CONCLUSIONS

MS medium supplemented with 0.08% colchicine for 24 h allowed to obtain *P. alkekengi* tetraploides. However, the tetraploid seedlings presented fewer leaves, smaller fruits and fewer seeds.

REFERENCES

ACANDA, Y. et al. Highly efficient in vitro tetraploid plant production via colchicine treatment using embryogenic suspension cultures in grapevine (*Vitis vinifera* cv. Mencía). **Plant Cell, Tissue and Organ Culture**, v. 123, n. 3, p. 547-555, September, 2015.

ACCUWHEATHER. Disponível em: <www.accuweather.com>. Acesso em: 25 nov. 2016.

ARVANITIS L. et al. Novel antagonistic interactions associated with plant polyploidization influence trait selection and habitat preference. **Ecology letters**, v. 13, n. 3, p. 330-337, January, 2010.

BADR, A. et al. Chromosomal criteria and taxonomic relationships in the Solanaceae. **Cytologia**, v. 62, n. 2, p. 103-113, June, 1997.

DANTAS, A. A. A. et al. Climatic classification and tendencies in Lavras region, MG. **Ciênc. Agrotec.** v. 31, n. 6, p. 1862-1866, Nov. 2007.

DOLEŽEL, J.; BINAROVA, P.; LUCRETTI, S. Analysis of nuclear DNA content in plant cells by flow cytometry. **Biologia Plantarum**, v. 31, n. 2, p. 113-120, March, 1989.

FERREIRA, E. B.; CAVALCANTI, P. P.; NOGUEIRA D. A. **ExpDes.pt**: Pacote Experimental Designs (brazilian portuguese). R package version 1.1.2. 2013. Disponível em: <https://cran.r-project.org/web/packages/ExpDes.pt /index.html>. Acesso em: 14 fev. 2017.

GUERRA, M.; SOUZA, M.J. **Como observar cromossomos: um guia de técnicas em citogenética vegetal, animal e humana**. Ribeirão Preto: Ed. FUNPEC, 2002. 131 p.

HONG, J. M. Anti-inflammatory activities of *Physalis alkekengi* var. franchetii extract through the inhibition of MMP-9 and AP-1 activation. **Immunobiology**, v. 220, n. 1, p. 1 - 9, January, 2015.

JESUS-GONZALEZ, L. WEATHERS, P. J. Tetraploid *Artemisia annua* hairy roots produce more artemisinin than diploids. **Plant Cell Reports**, v. 21, n. 8, p. 809-813, April, 2003.

JOHANSEN, D. A. **Plant microtechnique**. 2 ed. New York: Mc Graw-Hill, 1940. 523 p.

LIBERATO, S. et al. Cytogenetic of *Physalis peruviana* L., and *Physalis floridana* Rydb. Genotypes with differential response to *Fusarium oxysporum*. **Corpoica Ciencia y Tecnología Agropecuaria**, v. 15, n. 1, p. 51-61, January, 2014.

LIU, S. et al. In vitro induced tetraploid of *Dendranthema nankingense* (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. **Scientia Horticulturae**, v. 127, n. 3, p. 411-419, January, 2011.

MACEDO, W. R. et al. Unravelling the physiologic and metabolic action of thiamethoxam on rice plants. **Pesticide Biochemistry and Physiology**, v. 107, n. 2, p. 244 - 249, October, 2013.

NAKAMURA, N. et al. Development of tetraploid Chinese lantern (Physalis alkekengi L.var. franchetii Makino) and its characteristics. **Hort. Res. (Japan)**, v. 6, n. 3, p. 341-345, January, 2007.

OSORIO-GUARÍN et al. Association analysis for disease resistance to *Fusarium oxysporum* in cape gooseberry (*Physalis peruviana* L). **BMC Genomics**, v. 17, n. 248, n. p., March, 2016.

OSSANI, P. C.; CIRILLO, M. A.; **MVar.pt**: Analise multivariada (brazilian portuguese). 2017. R package version 1.9.8. Disponível em: <https://cran.r-project.org/web/packages/MVar.pt/index.html>. Acesso em: 10 fev. 2017.

POPA-MITROI et al. Study on behavior of *Physalis alkekengi* L. species in spontaneous flora and culture in order to evaluate its decorative quality**. South Westem Journal**, v. 3, n. 2, p. 185 - 202, March, 2012.

RAMIL, S.; SMITH, M.; Dodd, W. In vitro induction of banana autotetraploids by colchicine treatment of micropropagated diploids. **Aust J Bot**, v. 40, n. 6, p. 887-896, January, 1992.

RAMÍREZ-GODINA, F. et al. Yield and fruit quality evaluation in husk tomato autotetraploids (*Physalis ixocarpa*) and diploids. **Australian Journal of Crop Science**, v. 7, n. 7, p. 933-940, January, 2013.

R CORE TEAM 2017. R: A Language and Environment for Statistical Computing. **R Foundation for Statistical Computing**, Vienna, Austria. Disponível em: < http://www.R-project.org>. Acesso em: 05 mar. 2017.

SALE, B. et al. Tetraploid citrus rootstocks are more tolerant to salt stress than diploid. **Comptes Rendus Biologies**, v. 331, n. 9, p. 703-710, September, 2008.

SATTLER, M. C. et al. The polyploidy and its key role in plant breeding. **Planta**, v. 243, n. 2, p. 281-296, February, 2016.

SCHLAEPFER, D. R. et al. Why only tetraploid Solidago gigantea (Asteraceae) became invasive: a common garden comparison of ploidy levels. **Oecologia**, v. 163, n. 3, p. 661-673, July, 2010.

SCOPEL, W. et al. Extração de pigmentos foliares em plantas de canola. **Unoesc & Ciência - ACET**, v. 2, n. 1, p. 87-94, janeiro, 2011.

TORABZADEH, P.; PANAHI, P. Evaluation of antifungical activity of *Physalis alkekegi* L. extracts on Microsporum canis, Candida albicans, *Trichophyton mentagrophytes* and *Nocardia asteroids***. Middle-East Journal of Scientific Research**, v. 13, n. 7, p. 926 - 929, January, 2013.

WANG, R. A new combination in Alkekengi (Solanaceae) for the flora of China. **Phytotaxa**, v. 178, n. 1, p. 59 - 60, September, 2014.

ZHANG, C. Y. 1H NMR spectroscopy-guided isolation of new sucrose esters from Physalis alkekengi var. franchetii and their antibacterial activity. **Fitoterapia**, v. 114, n. 1, p. 138-143, September, 2016.

LI, X. et al. Physalins and withanolides from the fruits of Physalis alkekengi L. var. franchetii (Mast.) Makino and the inhibitory activities against human tumor cells. **Phytochemistry Letters**, v. 10, n. 1, p. 95 - 100, August, 2014.

MAJDI, M. et al. Induction of Tetraploidy to Feverfew (*Tanacetum parthenium* Schulz-Bip.): Morphological, Physiological, Cytological, and Phytochemical Changes. **HortScience**, v. 45, n. 1, p. 16-21, January, 2010.

MANZANEDA A. J. et al. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). **New phytologist**, v. 193, n. 3, p. 797-805, January, 2012.

NAKAMURA, N. et al. Development of tetraploid Chinese lantern (*Physalis alkekengi* L.var. franchetii Makino) and its characteristics. **Hort. Res. (Japan)**, v. 6, n. 3, p. 341-345, January, 2007.

OLIVEIRA, S. C. et al. In vitro polyploidization from shoot tips of Jatropha curcas L.: a biodiesel plant. **Plant Growth Regulation**, v. 69, n. 1, p. 79-86, January, 2013.

TAVAN, M. et al. In vitro polyploidy induction: changes in morphological, anatomical and phytochemical characteristics of *Thymus persicus* (Lamiaceae). **Plant Cell Tiss Organ Cult**, v. 122, n. 3, p. 573-583, May, 2015.

ZHANG, M. et al. Genome physical mapping of polyploids: a BIBAC physical map of cultivated tetraploid cotton, *Gossypium hirsutum* L**. PLoS One**, v. 7, n. 3, p. e33644, March, 2012.