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The influence exerted by LEDs and fluorescent tubes, of different colors, on regenerative processes and morphogenesis of *Rebutia heliosa in vitro* cultures

Influența exercitată de Led-uri și tuburile fluorescente, de diferite culori, asupra proceselor regenerative și a morfogenezei la culturile *in vitro* de *Rebutia heliosa*

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ABSTRACT

The paper traces how the light source and light color influence the regenerative processes and morphogenesis *in vitro* cultures of *Rebutia heliosa*. To establish the vitroculture of *R. heliosa*, some explants from young plants, either buds or rounds cut from young stems and cross-sectioned were considered. The culture medium consisted of: macroelements and Fe EDTA Murashige-Skoog (1962), microelements Heller (1953), a mineral mixture to which the following vitamins were added: pyridoxine HCl, thiamine HCl and nicotinic acid (each 1 mg/l, each), m-inositol – 100 mg/l, sucrose – 20 g/l and agar-agar 7 g/l, without growth phytoregulators.The variable element was the lighting source. Fluorescent tubes and LEDs (monochrome but differently coloured) have been used: blue (λ = 470 nm), yellow (λ = 580 nm), green (λ = 540 nm), red (λ = 670 nm) as well as LED`s emitting white light (λ = 510 nm) at a luminous intensity of 1000 lux. The evolution of *R. heliosa* vitro cultures was monitored for 90 days and analyzed their reaction variability. Based on the comparative study, the following can be stated: the light of fluorescent tubes is more suitable for the morphogenesis of *R. heliosa* vitroplants, but the processes of regeneration and organogenesis are directly influenced by the source and color of light influenced, thus rhizogenesis and caulogenesis is favored by the presence of green and red light emitted by LEDs, while the white and yellow light of fluorescent tubes favors the caulogenesis and callusogenesis processes.

Keywords: callusogenesis processes, fluorescent tubes, LEDs, roots, young stem

REZUMAT

Lucrarea urmărește modul în care sursa de iluminare și culoarea luminii influențează procesele regenerative și morfogeneza în vitroculturile de *R. heliosa*. Pentru a înființa vitrocultura de *R. heliosa* am prelevat explante de la plante tinere, fie mugurași, fie rondele decupate din tulpinile tinere și secționate transversal. Mediul de cultură utilizat de noi a constat din: macroe-lemente şi Fe EDTA Murashige-Skoog (1962), microelemente Heller (1953), amestec mineral la care au fost adăugate vitaminele: piridoxină HCl, tiamină HCl şi acid nicotinic (câte 1 mg/l, fiecare), m-inozitol – 100 mg/l, zaharoză – 20 g/l şi agar-agar 7 g/l, fără fitoregulatori de creștere. Variabila este reprezentată de sursa de iluminare, tuburi fluorescente și LED-uri (monocrome și diferit colorate): albastru (λ = 470nm), galben (λ = 580nm), verde (λ =

540nm), roșu (λ = 670nm) și LED-uri care emit lumină albă (λ = 510nm) la o intensitate luminoasă de 1000 lux. Am urmărit timp de 90 de zile evoluţia vitroculturilor de *R. heliosa* și am analizat variabilitatea de reacţie a acestora iar pe baza studiului comparativ putem afirma următoarele: lumina tuburilor fluorecente este mai potrivită pentru morfogeneza vitroplantulelor de *R. heliosa*, dar procesele de regenerare și organogeză sunt influențate în mod direct de sursa și culoarea luminii, astfel rizogeneza și caulogeneza este favorizează de prezența luminii verde și roșie emisă de LED-uri în timp ce lumina albă și galbenă a tuburilor fluorescente avantajează procesele de caulogeneză și calusogeneză.

Cuvinte-cheie: procesul de calusare, tuburi fluorescente, LED-uri, rădăcini, tulpini tinere

INTRODUCTION

Cacti are plants that belong to the *Cactaceae* family, a family that includes over 2000 species native to America and mostly distributed in arid, semi-arid and desert regions of the planet (Casas and Barbera, 2002). Most of the cactus species are endemic, being found in Argentina, Peru, Bolivia, Chile, Costa Rica and Mexico where approximately 80% of the genera and species are concentrated (Ortega-Baes et al., 2010). The current experiment has considered the plants of *R. heliosa*, a small cactus native to Bolivia, particularly valued for its flowers and widely marketed as a houseplant.

The conventional propagation of cacti is an expensive and often insufficiently fast method to the market demand, thus *in vitro* propagation is an alternative to classical propagation allowing the identical reproduction of the selected parents (Chen et al., 2002), in a period and limited space. The method also helps to conserve genetic resources and recover some endangered species.

Along their evolution, the superior plants have developed a wide range of photoreceptors that perceive and respond to spectrum stimuli, quantifying the quality and intensity of light, the direction of illumination and the duration of the light period (Winslow, 2002). They can use light to coordinate growth, development, and photosynthetic functions in a changing environment (Jonathan and Griffing, 2022) and are essential in orchestrating large-scale changes in gene expression to modulate photomorphogenesis (Franklin and Quail, 2010).

The success of *in vitro* cultures is dependent on parameters, but one of the decisive factors in controlling the processes of regeneration and morphogenesis is light. The traditional light source used for *in vitro* propagation is fluorescent lamps (Lin et al., 2011) which in addition to the significant consumption of electricity (Fang et al, 2004) also have the disadvantage of emitting a large amount of heat in the growth space which can be a stress trigger at the level of the inoculum. This fact imposed the need to find efficient alternative sources of lighting (Loberant and Arie, 2010), a versatile technology to remove these inconveniences. This proved to be the use of light-emitting diodes (LEDs) as a life support source and a potential alternative for *in vitro* plant growth and development (Yeh and Chung, 2009; Nhut and Nam, 2010).

Compared to the classic lighting system with fluorescent lamps, LEDs have the following advantages: they are more economical; they have small dimensions and relatively cold emission; they have wavelength specificity which makes the photosynthesis process more efficient (Armando et al., 2009); they have wavelength specificity; they allow independent control and precise manipulation of each spectral interval (Folta, 2004), thus the growth and development of cell cultures, tissues and plant organs can be controlled by triggering physiological reactions (Briggs and Olney 2001; Kurulcik et al., 2008); they are reliable over time; they emit light of a specific color depending on the phase needed to be induced; they can be focused on the target; they can be dimmed or pulsed, which increases the energy efficiency by 30% and protects the environment with low CO_2 emissions (Schindler and Lee 2010). For this reason, it is important to determine the amount in which photons with a certain wavelength induce economic growth and plant development.

In specialized literature, it is shown that the green light of the LEDs positively influences the number of newly formed stems in species of *Prunus* (Li et al., 2017) and *Gerbera jamesonii* (Gabryszewska and Rudnicki, 1995). Red LED light is associated with the induction of callusogenesis processes in *Anthurium andreanum* and *Hylocereus costaricensis* (Budiarto, 2010; Winson et al., 2021) and rhizogenesis in *Pinus* species (Merkle et al., 2005) respectively *Tripterospermum japonicum* (Moon et al., 2006) but also with the growth of *Oncidium* plants (Mengxi et al., 2011). *In vitro* cultures of *Gerbera jamesonii* higher rates of shoots regeneration, rooting and vegetative growth were observed in plants grown under blue and red LED light. (Lim et al., 2023)

Vitrocultures illuminated with yellow LEDs positively influence morphogenesis in *Prunus domestica* (Muleo et al., 2001) while the blue color of LEDs positively influences the dimensions of cauline neoformations in *Ficus benjamina* (Werbrouck et al., 2012) but inhibits the formation of roots in *Tripterospermum japonicum* (Moon et al., 2006) and in *Pinus* species (Merkle et al., 2005) but also of callus in *Anthurium andreanum* and *Hylocereus costaricensis* (Budiarto, 2010; Winson et al., 2021). White LED light is favorable for regeneration and organogenesis processes *in vitro* seedlings of *Oncidium tigrinum* and *Laelia autumnalis* (Martha et al., 2016). Therefore, detailed studies on the interaction between LEDs, their degrading-promoting effects and the stability of plant proliferation are required for each species. A careful management of these factors can ultimately lead to a more successful *in vitro* propagation (Cavallaro et al., 2023).

The necessity and novelty of this research start from the analysis of the specialized literature and from finding that most of the published studies have been focused on the impact of red and blue light (as well as their combination, usually in a 1:1 ratio) on the development of culture *in vitro* of ornamental plants (Werbrouck et al., 2012). This research was carried out to determine the influence that light of different wavelengths emitted by fluorescent tubes has on morphological processes compared to that emitted by monochrome LEDs of different colors (blue, yellow, green, red, white), respectively rhizogenesis, caulogenesis and callusogenesis of the *in vitro* culture of *R. heliosa*.

MATERIALS AND METHODS

To initiate the vitro cultures of *R. heliosa*, young and healthy plants able to ensure the objectivity of the results have been used considering that the organogenetic potential of immature tissues is superior compared to those from cacti older than five years, whose "*in vitro*", cultivation from this point of view, it could fail (Papafotiou et al., 2001).

It is known that parts taken from different areas of the same plant grown "*in vitro*" give different reactions (Cachiță et al., 2004), in the current experiment the reactivity of the following categories of explants was monitored: buds (Figure 1-1a) and the rounds cut from the cross-sectioned young stems (Figure 1-2b). The portioned and dimensioned young stems form explants from either the middle (Figure 1-2d) or the side of the disc (Figure 1-2c). The buds harvested with a diameter larger than about 1 cm were shaped so that the explants did not exceed the established dimensions; all buds, regardless of size, were shaped by removing the basal area (Figure 1-2b). In cacti grown *in vitro*, the number of areoles per explant is very important because regeneration is achieved following the meristematic activity of cells in the areoles (Lema-Rumińska and Kulus, 2014).

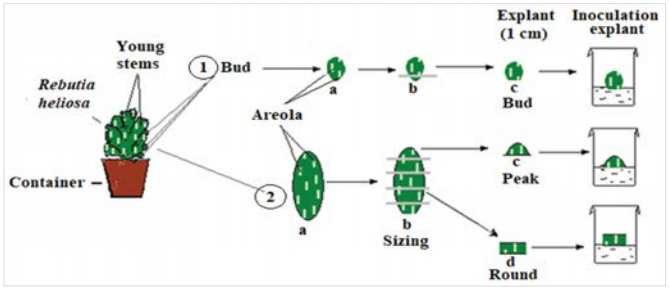

Figure 1. Schematic representation of the method of explanting the fragments that were inoculated on aseptic media (where: dimensioning of buds and obtaining spheroidal discs of *R. heliosa*, sectioned from young stems)

The *R. heliosa* explants were harvested after asepticizing the plant material in a hood with laminar, horizontal, sterile airflow, in operation, the necrotic parts were detached from the fragments detached from stems and they were sized to about 1 cm in length, 0.5 cm thick and a diameter of 0.5 - 1.5 cm, shaped so that each has 2 - 3 areolae.

The nutrient medium used in the "*in vitro*" culture of cacti is that proposed by Murashige and Skoog (1962), abbreviated MS, considered to have a complete and balanced chemical composition. This medium was also widely used in "*in vitro*" cultivation of cactus in the experiments of Estrada Luna et al. (2008), Angulo-Bejarano and Paredes-López (2011), Ferreira et al. (2021), and Mabrouk et al. (2021). The present experiment uses the MS to which vitamins were added: pyridoxine HCl, thiamine HCl and nicotinic acid (1 mg/l each), m-inositol – 100 mg/l, sucrose – 20 g/l and agar-agar 7 g/l, without growth phytoregulators.

After preparing the culture medium, 5 ml of MS media was introduced in each bottle with a capacity of 15 ml. To asepticize the plant material was submerged for one minute in 96° ethyl alcohol, the process being followed by covering it, for 20 minutes, with a 0.8% sodium hypochlorite solution, in a mixture with water, in a ratio of 1:2, in which three drops of Tween 20 were added, as a surfactant (Cachiţă et al., 2004), during which they were stirred continuously. The combination is considered effective in neutralizing the microbiota in cacti (Estrada Luna et al., 2008; Bhau, 2015). Their areolas are hosts for a large number of microorganisms (Garcia-Saucedo et al., 2005). To remove the chlorine, the next step consisted in five consecutive rinses with sterile distilled water, for five minutes. The inoculation of the explants was carried out in the aseptic room, in the hood with a laminar flow of sterile air.

After inoculation, in all experimental phases, the containers with explants were transferred for incubation and growth in special chambers where the variable consisted of illumination with light sources of different wavelengths and colors. For the control sample, V_0 – values considered 100%, containers with *R. heliosa* explant were placed on shelves and exposed to a growth regime that consisted of the following conditions: white light emitted by fluorescent tubes (λ = 400 nm), at a light intensity of 1700 lux, located at a distance of 33 cm from the surface of the culture medium on which the explants were placed; the photoperiod regime was of 16 hours.

For the illumination of *R. heliosa* explants with monochrome fluorescent tubes, emitting colored lights and different wavelengths (blue, yellow, green, red). In parallel, vitro cultures of the same type were organized, but lit in growth chambers with monochrome LEDs of different colors (blue, yellow, green, red and white) which allowed the lighting devices, during their operation, to be positioned one above each culture container. Inside the growth chambers, the light intensity was 1000 lux regardless of the color of the LEDs, the photoperiod was 16 hours of light out of 24 hours, and a temperature that oscillated between 24 °C and 27 °C; 15 vials were inserted in each box.

Depending on the light source to which the inocula were exposed, two experimental series were obtained, as follows:

S1 - viticulture artificially illuminated with fluorescent tubes generating lights of various colors: V_0 – lighting with white fluorescent tubes – control lot, (λ = 400 nm); N_1 – lighting with blue fluorescent tubes (λ = 470 nm); N_2 – lighting with yellow fluorescent tubes (λ = 580 nm); N_3 – lighting with green fluorescent tubes (λ = 540 nm); N_4 – lighting with red fluorescent tubes (λ = 670 nm);

S2 - glass cultures artificially lit with LEDs generating lights of various colors: V_0 – lighting with white fluorescent tubes – control lot, (λ = 400 nm); L_0 – lighting with LEDs emitting white light (λ = 510 nm); L_1 – lighting with LEDs emitting blue light (λ = 470 nm); L_2 – lighting with LEDs emitting yellow light (λ = 580 nm); L_3 – lighting with LEDs emitting green light (λ = 540 nm); L_4 – lighting with LEDs emitting red light (λ = 670 nm);

The reaction of the explants and the evolution of the *R. heliosa* explants were periodically monitored over a period of 90 days, the following parameters being evaluated: the survival percentage of the explants; the basal diameter of the stem (using the caliper); the number of kaulin neoformations; the diameter of the kauline neoformations; the number of unformed roots at the inoculum level (using graph paper); the length of the newly formed roots; the production of callus, if it existed and its dimensions (using a caliper, graph paper or ruler, as appropriate).

Design of Experiment (DOE). The DOE considered two factors and the interaction between them: Factor 1, LightSOURCE, with two levels: TF, tungsten light source type and LED, light emitting diode light source; Factor 2, LightCOLOUR, with five levels: W for white light colour, B for blue light colour, G for green light colour, R for red light colour and Y for yellow light colour; Interaction Factor, LightSOURCE* LightCOLOUR, with 10 levels: TF_W, TF_B, TF_G, TF_R, TF_Y, LED_W, LED_B, LED_G, LED_R and LED_Y.

Statistical analysis

All data was subjected to two-way ANOVA (P = 0.05) and pairwise comparisons were done with the post-hoc Ducan test (P = 0.05). The comparisons covered all three analyzed factors, including the interaction one, the results being displayed as means ± standard deviation and the sampling number being prescribed correspondingly.

The multivariate analysis sequence was performed to emphasize the variable grouping and sample clustering. The sequence consisted of PCA (Principal Component Analysis), LDA (Linear discriminant Analysis), and MANOVA (P = 0.05) (Multivariate ANalysis Of VAriance). From these multivariate methods, only MANOVA performs statistical significance for pairwise comparisons of the multivariate sample's profiles with 95% accuracy (i.e., P = 0.05). In this way, the multivariate results have statistical accuracy. The LDA ordination was mentioned because its outputs (i.e., canonical coordinates of the samples) were considered as input for the MANOVA test. Also, the LDA input data represented the principal coordinates of the samples calculated with the PCA method.

All the statistical calculations and graphical representations were done with custom-made programs developed with MATLAB 2023a v9.14.0.2337262 CWL (The MathWorks Inc., 1 Apple Hill Drive, Natick, MA, USA).

RESULTS AND DISCUSSION

After five days from inoculation, the number of containers eliminated due to infections did not exceed 14%, in any of the experimental variants, which represents a good percentage considering that one of the constraints of *in vitro* culture is fungal contamination and bacteria of the biological material used, even more so in cacti that have located different resistance structures of some fungi at the level of the areoles (Garcés, 2003).

After 90 days from the beginning of this experiment, the results demonstrate the beneficial influence that the light emitted by LEDs of different colors and wavelengths has on the survival percentage of *R. heliosa* explants. Compared to the control V_0 (*in vitro* culture illuminated with white fluorescent tubes) the variants of the S2 series (*in vitro* cultures artificially illuminated with light emitted by LEDs) recorded a survival percentage, at the level of explants, between 89-97%, while in the S1 series (vitro cultures artificially illuminated with fluorescent tubes) this parameter represented only 57-63%.

The comparisons between the factors' levels for the analyzed parameters are presented in Tables 1–3, including the interaction factor. However, for a better understanding of the results, they were graphically represented as main effects graphs (Figure 2 a, b, c, d, e, f).

The main effects graphs consist of three graphical panels with the mean values represented corresponding to the levels of each factor, including the interaction factor which was represented as complementary information but not for effects comparison.

Table 1. Mean results of the physiological parameters calculated for factor LightSOURCE levels with two-way ANOVA

Source of light	newSTEM	dSTEM	newROOT	LROOT	newCAL	dCAL
TF	2.415 ± 1.398a	0.807^{a} ± 0.333	0.297^{a} ± 0.512	0.297^{a} ± 0.512	1.847^{a} ± 1.344	1.847^{a} ± 1.344
LED	2.112^{b} ± 1.826	0.787^{a} ± 0.501	0.078^{b} ± 0.299	0.032^{b} ± 0.130	1.707^{a} ± 1.433	0.798^{b} ± 0.560

Note: Across columns for each variable, different letters that accompany the mean values denote statistically significant differences between the samples' means. Pairwise comparisons were done with the Duncan *post-hoc* test (P = 0.05). Results are expressed as mean ± standard deviation (N = 118 for TF and N = 116 for LED). Where: TF - fluorescent tubes; LEDs; newSTEM - newly formed stems; dSTEM - diameter of newly formed stems; newROOT - newly formed roots, LROOT - length of roots; newCAL - callus formed; dCAL - diameter of calluses.

The horizontal dotted line denotes the grand mean over all the samples of the analyzed parameter. In this way, one can compare the main effects between the parameters.

All the parameters, excluding the diameter of calluses – dCAL, have the LightCOLOUR as the dominant (i.e., main) effect. For the newSTEM, the difference is higher enough, visually, these responses of *R. heliosa* vitroplants to stimuli related to the color of light confirm the results from the specialized literature that demonstrate the positive effect on the caulogenesis process in explants grown in the red or green light, thus the green light emitted by LEDs causes an increase in the number and dimensions of the ramifications *in vitro* cultures of lettuce (Pardo et al., 2014) and bell pepper (Casierra-Posada et al., 2014).

The same phenomenon also occurs under red light *in vitro* cultures of *Myrtus cummunis* L. (Cioć et al., 2018), *Oncidium* (Liu M. et al., 2011) or *Ficus benjamina* (Werbrouck, et al., 2012), the beneficial influence of red light in the generation of callus is also recognized, for example in vitroplants of *Anthurium andreanum* (Budiarto, 2010).

Factor LightSOURCE denotes the TF with the mean values higher than the grand means for all the analyzed parameters. These results are in contradiction with those presented by Nowakowska et al. (2023) who in *Helleborus 'Molly's White'* obtained the highest multiplication rate *in vitro* cultures grown under LEDs, compared to those grown under fluorescent lamps.

The results presented here suggest that the different responses of *in vitro* culture plants to the light source and color require the need for different lighting conditions depending on the species and the stage of plant development, and to achieve the objectives related to regeneration and organogenesis whose effect on each *in vitro* culture must be studied separately (Kim et al., 2004a; Poudel et al., 2008).

Table 2. Mean results of the physiological parameters calculated for factor LightCOLOUR levels with two-way ANOVA

Color of light	newSTEM	dSTEM	newROOT	LROOT	newCAL	dCAL
W	1.863^{c} ± 1.456	0.745^{bc} ± 0.381	0.137^{bc} ± 0.348	0.137^{bc} ± 0.348	2.510^{a} ± 1.642	1.759^{a} ± 1.272
B	0.909^{d} ± 0.709	0.652^{c} ± 0.421	0.205^{ab} ± 0.462	0.084^{cd} ± 0.202	1.591^{b} ± 0.996	1.264^{bc} ± 0.980
G	3.083^{b} ± 1.528	0.846^{b} ± 0.485	0.354^{a} ± 0.565	0.354^{a} ± 0.565	1.854^{b} ± 1.031	1.279^{bc} ± 0.807
R	3.935^{a} ± 1.143	1.030^{a} ± 0.303	0.239^{ab} ± 0.480	0.239^{ab} ± 0.480	1.457^{b} ± 1.089	0.911^{c} ± 0.566
Y	1.467^{c} ± 0.991	0.707^{bc} ± 0.419	0.000^{c} ± 0.000	0.000^{d} ± 0.000	1.378^{b} ± 1.696	1.378^{b} ± 1.696

Note: Across columns for each variable, different letters that accompany the mean values denote statistically significant differences between the samples' means. Pairwise comparisons were done with the Duncan *post-hoc* test (P = 0.05). Results are expressed as mean ± standard deviation (N = 51 for W, N = 44 for B, N = 48 for G, N = 46 for R and N = 45 for LED). Where; W - white light source; B - blue light source; G - green light source; R - red light source; Y - yellow light source; newSTEM - newly formed stems; dSTEM-diameter of newly formed stems; newROOT - newly formed roots, LROOT - length of roots; newCAL - callus formed; dCAL - diameter of calluses.

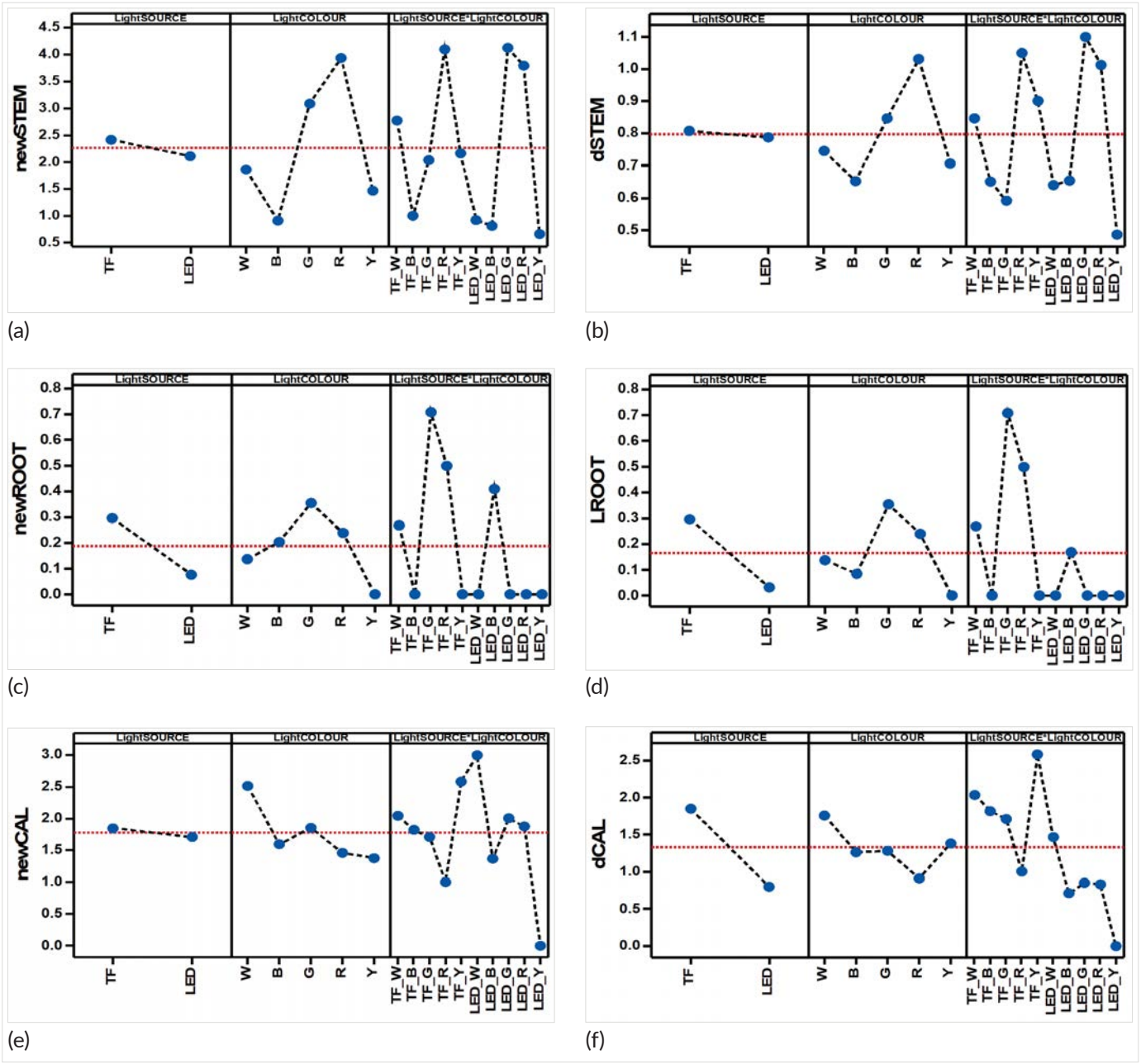

Figure 2. Main effects of the physiological parameters for the DOE factors: LightSOURCE, LightCOLOUR and LightSOURCE*LightCOLOUR (the horizontal dotted red line denotes the grand mean for the corresponding parameter)

In vitro culture of *R. heliosa* for the LROOT, dROOT and dCAL, the difference between TF and LED effects (i.e., between the mean values) are higher than for the other parameters – newSTEM, dSTEM and newCAL. The LightCOLOUR factor levels G and R perform highest mean values and as a consequence the dominant effects for the newSTEM, dSTEM, newROOT and LROOT. These results are similar to those published by Toshinari et al. (2011) *in vitro* cultures of *Bletilla ochracea*, *Anthurium andreanum* (Martinez-Estrada et al., 2016) *Gossypium hirsutum* (Li et al., 2010). In the meantime, the W level performs the highest mean values for the newCAL and dCAL parameters, denoting it with the dominant effect. The last note is to mention the LightSOURCE*LightCOLOUR levels, for each parameter, that have mean values higher than the corresponding grand means, for the newSTEM.

Table 3 shows that the T_W and TF_R generate dominant effects with high frequency over all the parameters. Furthermore, the newCAL parameter is the most sensitive to LightSOURCE and LightCOLOUR changes; it is followed by the dSTEM and dCAL. Because of the complex pattern obtained in Figure 5, the multivariate analysis was used to generate the sample clusters and variable (i.e., parameter) grouping to perform the cluster discrimination.

Multivariate analysis considered first the PCA method to obtain the summarizing statistical information and the biplot's graphical representation. In a feed before step, it is mentioned that the points spatially distributions: in the PC1 and PC2 axes frame including the newROOT and LROOT parameters in PCA calculations and without them, are different (Figure 3).

The latter one is more dispersed and is usually obtained in PCA analysis. The first one (i.e., that includes newROOT and LROOT parameters) performs a high peak density located near the axes frame origin (see the kernel density plot in Figure 3 d and biplot in Figure 3 c). This density peak is 1.6 times higher than one obtained from PCA analysis without newROOT and LROOT parameters (Figure 3 b).

These results are noticeable from the corresponding biplots in Figures 3 a and b, and the parameters newROOT and LROOT were left outside the PCA analysis. A supplementary fact, that generates these results, is that the newROOT and LROOT parameters perform numerical results for all samples that have binomial behaviour; in comparison, the other parameters generate multinomial to contiguous behavioural results for all the analyzed samples.

Biplots in Figure 4 show that the TF-related samples provide a wider range over the first three principal axes than the LED-related samples. The newCAL and dCAL parameters vectors are positively strongly correlated with the first main component, PC1, due to the small angles between them and the PC1. As a consequence, these parameters give the maximal variance for the DOE, and also, form a variables group. The other parameters involved in PCA calculations, newSTEM and dSTEM

Table 3. Mean results of the physiological parameters calculated for the interaction factor LightSOURCE*LightCOLOUR levels with two-way ANOVA

Source of light/color	newSTEM	dSTEM	newROOT	LROOT	newCAL	dCAL
TF_W	$2.769^{b} \pm 1.423$	$0.846^{bc} \pm 0.296$	$0.269^{c} \pm 0.452$	$0.269^{c} \pm 0.452$	$2.038^{bc} \pm 1.708$	$2.038^{b} \pm 1.708$
TF_B	$1.000^{d} \pm 0.816$	$0.650^{cd} \pm 0.418$	$0.000^{d} \pm 0.000$	$0.000^{d} \pm 0.000$	$1.818^{c} \pm 1.097$	$1.818^{bc} \pm 1.097$
TF_G	$2.042^{c} \pm 1.233$	$0.592^{d} \pm 0.298$	$0.708^{a} \pm 0.624$	$0.708^{a} \pm 0.624$	$1.708^{cd} \pm 0.908$	$1.708^{bc} \pm 0.908$
TF_R	$4.091^{a} \pm 0.610$	$1.050^{ab} \pm 0.211$	$0.500^{ab} \pm 0.598$	$0.500^{b} \pm 0.598$	$1.000^{d} \pm 0.756$	$1.000^{d} \pm 0.756$
TF_Y	$2.167^{c} \pm 0.565$	$0.900^{ab} \pm 0.202$	$0.000^{d} \pm 0.000$	$0.000^{d} \pm 0.000$	$2.583^{ab} \pm 1.501$	$2.583^{a} \pm 1.501$
LED_W	$0.920^{d} \pm 0.702$	$0.640^{cd} \pm 0.435$	$0.000^{d} \pm 0.000$	$0.000^{d} \pm 0.000$	$3.000^{a} \pm 1.443$	$1.468^{cd} \pm 1.443$
LED_B	$0.818^{d} \pm 0.588$	$0.655^{cd} \pm 0.433$	$0.409^{bc} \pm 0.590$	$0.168^{cd} \pm 0.263$	$1.364^{cd} \pm 0.848$	$0.709^{e} \pm 0.848$
LED_G	$4.125^{a} \pm 0.992$	$1.100^{a} \pm 0.507$	$0.000^{d} \pm 0.000$	$0.000^{d} \pm 0.000$	$2.000^{bc} \pm 1.142$	$0.850^{e} \pm 1.142$
LED_R	$3.792^{a} \pm 1.474$	$1.013^{ab} \pm 0.372$	$0.000^{d} \pm 0.000$	$0.000^{d} \pm 0.000$	$1.875^{bc} \pm 1.191$	$0.829^{e} \pm 1.191$
LED_Y	$0.667^{d} \pm 0.730$	$0.486^{d} \pm 0.492$	$0.000^{d} \pm 0.000$	$0.000^{d} \pm 0.000$	$0.000^{e} \pm 0.000$	$0.000^{f} \pm 0.000$

Note: Across columns for each variable, different letters that accompany the mean values denote statistically significant differences between the samples' means. Pairwise comparisons were done with the Duncan *post-hoc* test ($P = 0.05$). Results are expressed as mean ± standard deviation (N = 25 for LED_W, N = 22 for LED_B, N = 24 for LED_G, N = 24 for LED_R, N = 21 for LED_Y, N = 26 for TF_W, N = 22 for TF_B, N = 24 for TF_G, N = 22 for TF_R and N = 24 for TF_Y). Where: TF_W - white fluorescent tubes; TF_B - blue fluorescent tubes; TF_G - green fluorescent tubes; TF_R - red fluorescent tubes; TF_Y - yellow fluorescent tubes; LED_W - white LEDs; LED_B - blue LEDs; LED_G - green LEDs; LED_R - red LEDs; LED_Y - yellow LEDs; newSTEM - newly formed stems; dSTEM - diameter of newly formed stems; newROOT - newly formed roots, LROOT - length of roots; newCAL - callus formed; dCAL - diameter of calluses.

consist of single variable groups. They have a strong positive correlation with the PC2 principal component (due to small angles between them and the PC2), but dSTEM is positively strongly correlated with PC3, meanwhile, the newSTEM is negatively correlated with the PC3.

The variable vector arrangement defines the samples' distribution. The samples: TF_W, TF_Y TF_B and LED_W are located "along" the PC1 axis and with mean values higher than the grand means for the newCAL and dCAL (Figure 2 e, f), thus these samples are located near the tips of the mentioned variables vectors. On the opposite direction, also along the PC1 axis, the following samples are located: LED_B, LED_Y, TF_R, LED_R and LED_G – these samples have mean values lower than the grand means for the newCAL and dCAL (Figure 2 e, f), thus they have a low abundance of newCAL and dCAL parameters.

It should be noted that surpassing the control V_0, the average values of the most callus/variant (2.58) and the largest average diameter (1.28 cm) obtained in *R. heliosa* vitroplants grown under the yellow light of fluorescent tubes while those grown under the same color LED light did not show callus.

Furthermore, from this sample group, the samples TF_R, LED_R and LED_G are located in the positive direction of the newSTEM and dSTEM vectors and with mean values higher than the grand means for the mentioned parameters (Figure 2 c and d). These results are similar to those presented by Monika Cioć et al. (2018) *in vitro* culture of *Myrtus cummunis*, in *Ficus benjamina* (Werbrouck et al., 2012) and *Rehmannia glutinosa* (Hahn et al., 2000) *in vitro* cultures grown under red light and the similar effect determined by light from green color in vitroculture of sugarcane (Maluta et al., 2013).

In this way, parameters newCAL and dCAL have simultaneously the highest abundance for the TF_W, LED_W and TF_Y samples. These results are similar to those reported by Murillo-Talavera et al. (2016), which show that the light emitted by white LEDs was favorable to the processes of regeneration and organogenesis in the vitroplant of *Oncidium tigrinum* and *Laelia autumnalis*.

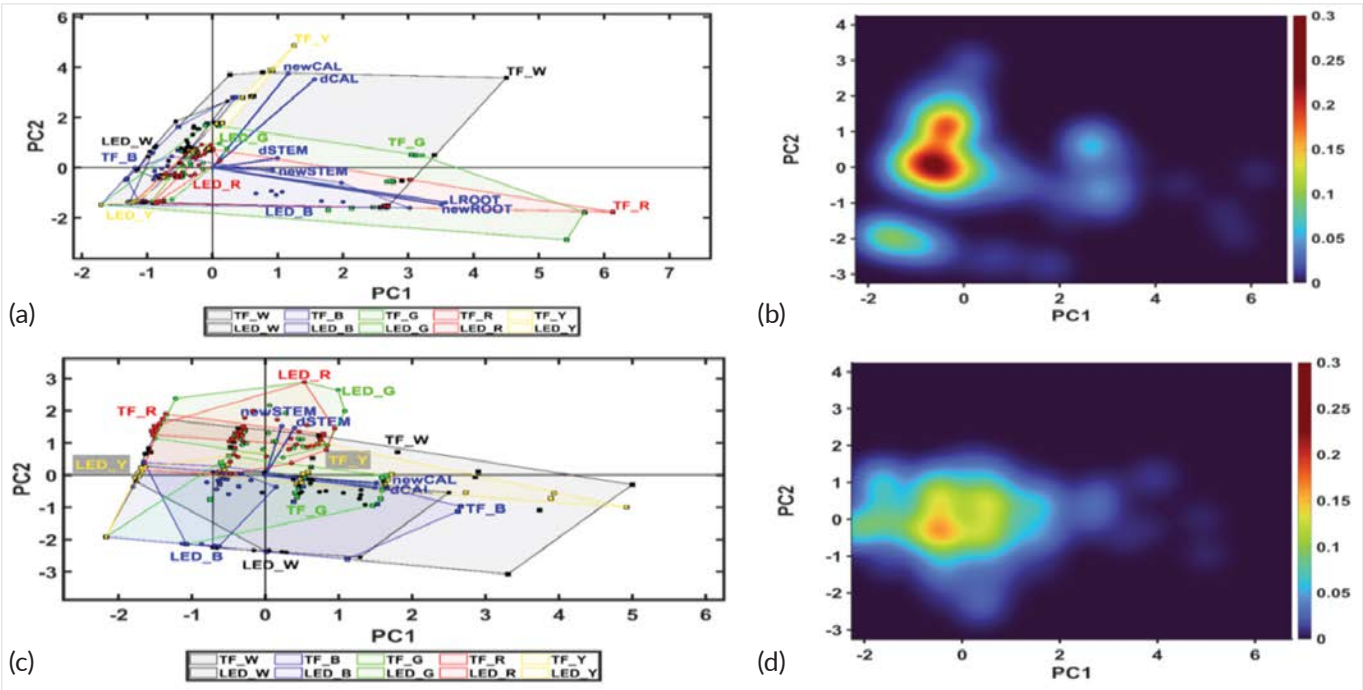

Figure 3. PCA results represented as biplots: (a) 2D representation for PC1 and PC2 principal components with newROOT and LROOT parameters; (c) 2D representation for PC1 and PC2 without newROOT and LROOT parameters; and as kernel density maps (b) for PC1 and PC2 principal components with newROOT and LROOT parameters; and (d) for PC1 and PC2 principal components without newROOT and LROOT parameters

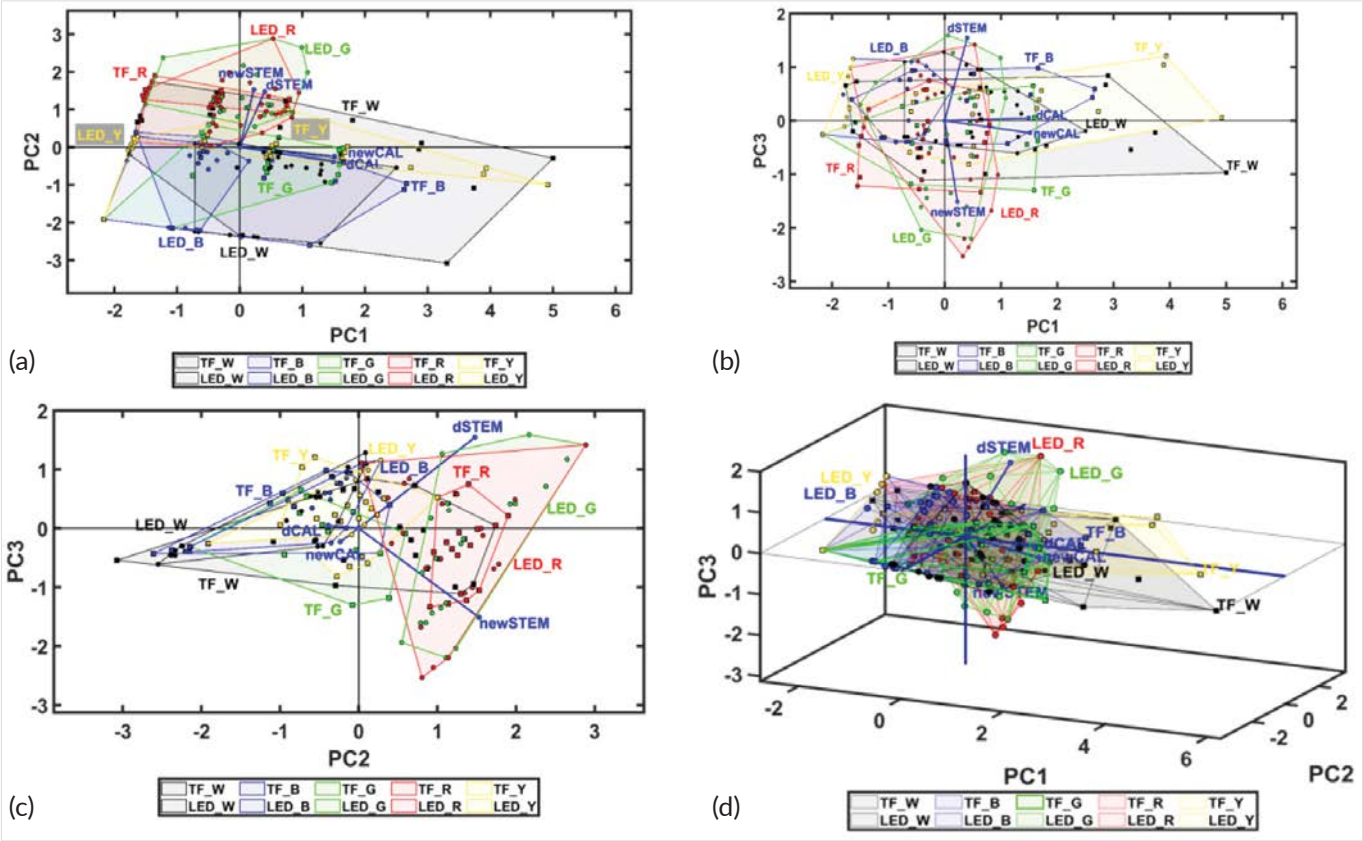

Figure 4. PCA results represented as biplots: (a) 2D representation for PC1 and PC2 principal components; (b) 2D representation for PC1 and PC3; (c) 2D representation for PC2 and PC3 principal components; and (d) 3D representation for PC1, PC2, and PC3

On the other hand, parameters newSTEM and dSTEM have simultaneously the highest abundance for TF_R, LED_R and LED_G samples. All the considered parameters in PCA calculations (newCAL, dCAL, newSTEM and dSTEM) don't have simultaneously highest abundance for any of the samples, but for technological reasons, the previous discussion provides the rationale that one can use to combine the light sources to have abundance for the desired parameter.

On the opposite direction, also along the PC2 axis, the following samples are located: LED_B and LED_Y and with mean values lower than the grand means for the mentioned parameters (Figure 2 c and d).

The PCA analysis emphasizes several samples that have the highest abundance for corresponding parameters, a fact that suggests possible clustering information that can be revealed. The multivariate methods for sample clustering are the LDA and MANOVA (P = 0.05). The canonical coordinates were used as input data for the MANOVA (P = 0.05) test that performed the statistical significances of samples pairwise comparisons at the 95% confidence level (i.e., P = 0.05). Also, the MANOVA test generated the clustering information presented as a dendrogram (Figure 5) and tabulated it.

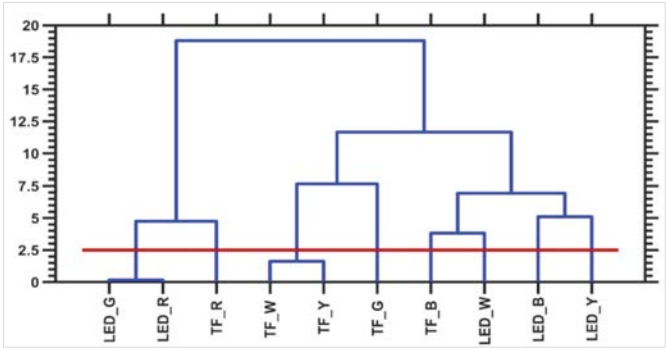

Figure 5. Clustering results from MANOVA (P = 0.05) and AHC as a dendrogram with the cut-off red line that generates the proper number of clusters

The sample clustering is, also, presented graphically in Figure 6. The multivariate clustering process concludes that there are two double clusters: one, that gathers samples LED_G and LED_R and the second, that gathers samples TF_W and TF_Y; the rest of the clusters are single clusters.

Consequently, it can be said that the results obtained for the *in vitro* cultures of *R. heliosa* are similar to those published in the specialized literature according to which light is a decisive factor in regulating the growth and development of plants *in vitro*, especially the light in the spectral region that is involved in the photomorphogen responses (Gupta and Jatothu, 2013).

At the level of the callus, the phenomenon of hyperhydria is manifested (Figure 7), which according to Pérez et al. (2002) represents a serious problem that limits the "*in vitro*" culture of cacti, a fact also mentioned by Souza et al. (2019) who reported a high percentage of hyperhydria, 88–100%, in *in vitro* cultures of *Opuntia stricta*. Wellens (2003) agrees, and believes that due to the frequency of hyperhydria *in vitro* cactus cultures, the aim is to obtain callus or somatic embryogenesis.

In the current experiment, as it has already been shown, the explants of *R. heliosa* were inoculated on basic media without hormonal input, the growth conditions presenting the light source, color and wavelength as variable. Probably the conditions imposed by the lighting of vitro cultures are the factor that triggered and maintained both the changes in morphological characters and the hyperhydria at the level of *R. heliosa* explants, a fact also noted by Saher et al. (2004) and Ht et al. (2011).

Based on the results, it can be concluded that the response of vitro cultures to the light and color source is very different and the effects of this stimulus on the processes of regeneration and morphogenesis must be studied individually. In short, it can be said that light intensity directly influences the processes of regeneration and organogenesis at the level of *R. heliosa* in vitroplants, because this factor affects the content of endogenous growth regulators (Manivannan et al., 2017). This aspect favors the possibility of using LEDs as an alternative and viable source to fluorescent tubes due to the adaptability of their wavelength to the needs of vitroplant photoreceptors.

Another problem faced in all experimental variants was the necrosis of *R. heliosa* explants (Figure 7), a manifestation due to the release of phenolic compounds, a phenomenon considered to be another problem of *in vitro* cultures in cacti (Ramirez-Malagon et al., 2007; Lema-Ruminska and Kulus, 2014).

Analyzing the images in the figures below (Figure 7), it can be seen that at the level of *R. heliosa* explants grown under the incidence of monochrome but variously colored light from fluorescent tubes or LEDs, a non-generalized phenomenon, but present in various forms, appeared spontaneously, accidentally of manifestation in all the

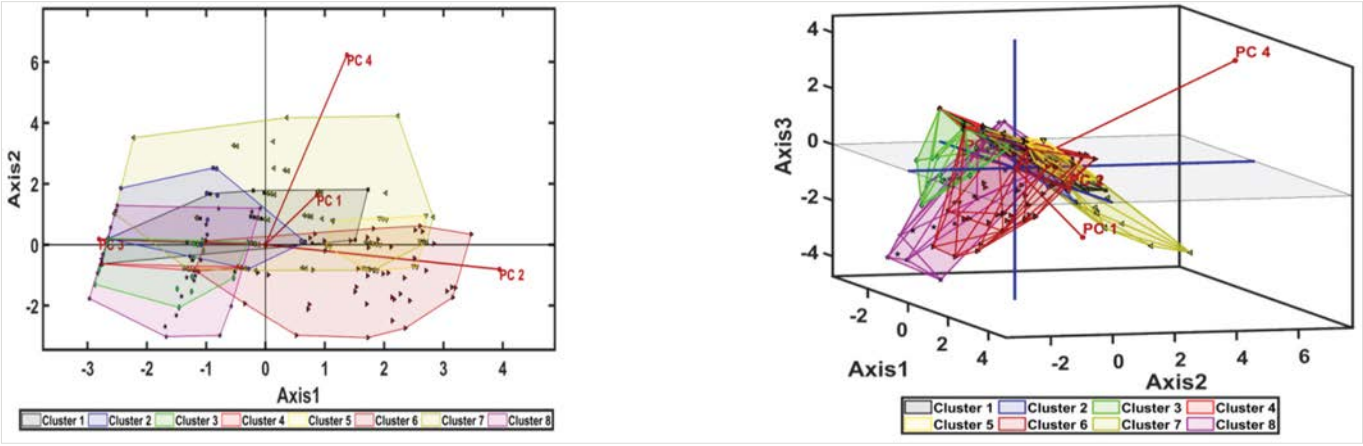

Figure 6. Clustering results of all 234 samples represented as LDA biplots (a – in 2D and b in 3D) – clusters are in different colours according to the information from the multivariate methods MANOVA (P = 0.05) and AHC

experimental variants studied; it is about changes in their external morphological characters, expressed in various ways: from the different disposition of the spines or the atypical shape of the stem to an intense proliferative activity at the level of the explant, which leads to the appearance of malformed forms, as a result of various suffered deformations.

The white light emitted by both fluorescent tubes (variant N_0) and LEDs (variant L_0) determined the induction of callusogenesis at the explant level. In the light of the fluorescent tubes (variant N_0) the generated callus was friable, greenish, located at the base of the cauline neoformations, which preserves the characteristics of the *R. heliosa* species in terms of the shape and color of the thorns (Figure 7- 1a, 1b); in the light of the LEDs (variant L_0) the callus was vitrified, with differently colored areas from green to white passing through different shades. Being abundant, it covered both the initial inoculum and a large part of the culture medium (Figure 7-1b).

Under the incidence of blue light emitted by both fluorescent tubes (variant N_1) and LEDs (variant L_1) it is noticeable that the initial inoculum is necrotic and at its level a compact, optically dense callus with a leathery appearance has been born, variously colored with areas in shades of pale green, whitish or yellow to brown (Figure 7-2a, 2b).

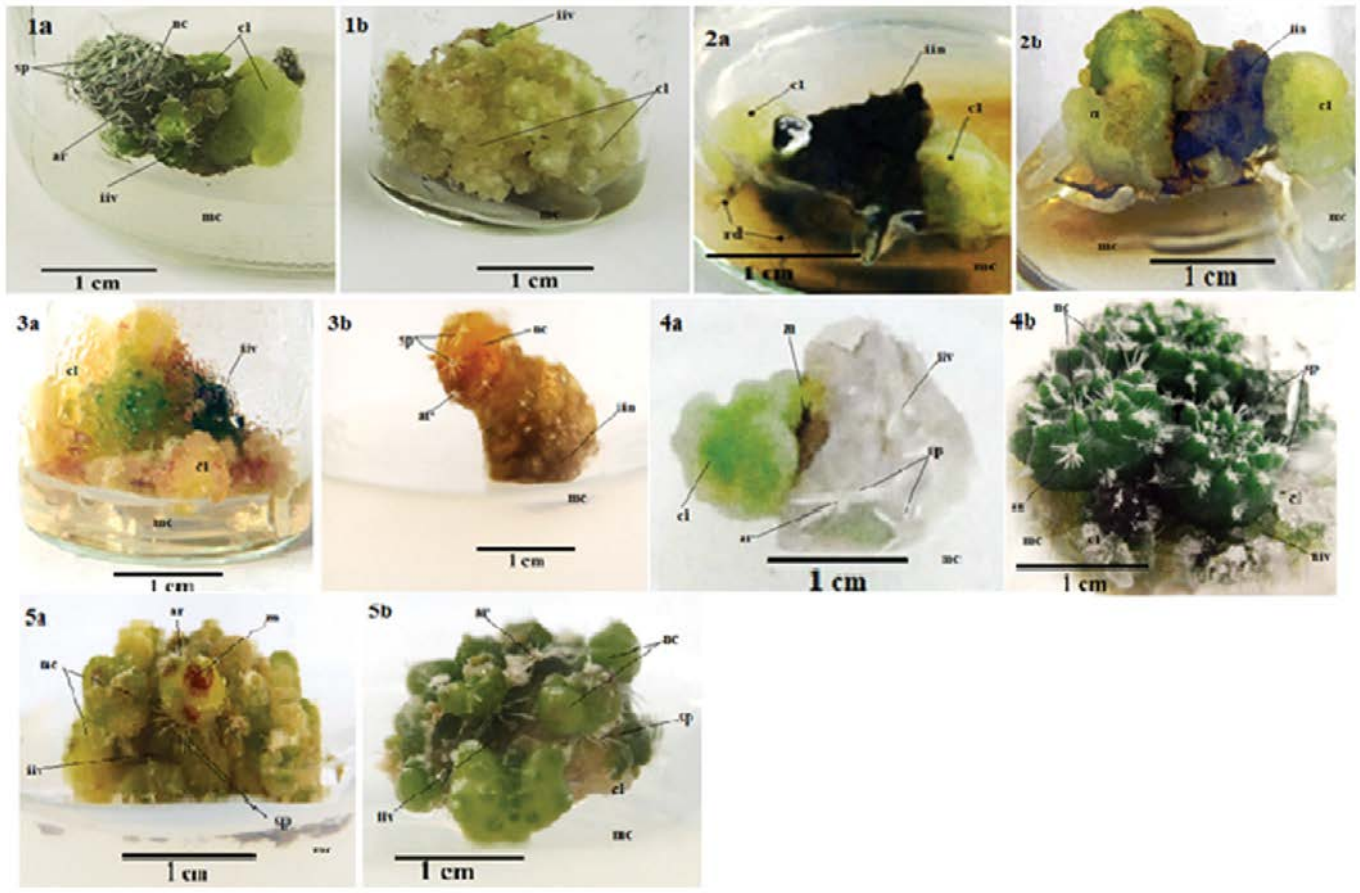

Figure 7. *R. heliosa* inoculums cultured on MS basal medium lacking growth regulators and illuminated with white light (Where: 1a–vitrocultures illuminated with white fluorescent tubes (V_0); 1b-vitro cultures illuminated with white LEDs (L_0); 2a–vitrocultures illuminated with blue fluorescent tubes (N_1); 2b-vitro cultures illuminated with blue LEDs (L_1); 3a-vitrocultures illuminated with yellow fluorescent tubes (N_2); 3b-vitro cultures illuminated with yellow LEDs (L_2); 4a-vitrocultures illuminated with green fluorescent tubes (N_3); 4b-vitrocultures illuminated with green LEDs (L_3); 5a-vitrocultures illuminated with red fluorescent tubes (N_4); 5b-vitro cultures illuminated with red LEDs (L_4); (iiv - viable initial inoculum; mc - culture medium; nc - cauline neoformation; ar - areoles; sp - spines; cl - callus; zn - necrotic zone)

The yellow light acted differently depending on the emission source. Under the incidence of fluorescent tubes (variant N_2) the explants became necrotic, and the callus generated was abundant with a glassy - vitrified - cream to tan color appearance, lacking morphogenesis (Figure 7-3a). The explants lost their spines *in vitro* cultures exposed to LED light (variant L_2), the areoles being small without hairs or glochids. However, at the level of the newly-formed bud, the spines had the form of a comb, characteristic of the *Rebutia heliosa* species (Figure 7-3b). Both the initial inoculum and the new buds changed their color becoming greenish-brown.

At the level of the explants exposed to the green light emitted by the fluorescent tubes (variant N_3), a yellow-green callus was generated with white edges, with a velvety appearance. It should be noted that the initial inoculum changed its color becoming white, but also kept the characteristic disposition of the spines (Figure 7-4a). Exposed to LEDs of the same color, the explants were distinguished by an intense proliferative activity, which resulted in a large number of new buds, small in size and intense green in color, with one areole in the apical area; at the base they had areoles surrounded by comb-shaped thorns (Figure 7-4b).

The response of the explants grown both to the red light of fluorescent tubes (variant N_4) and LEDs (variant L_4) was also an intense proliferative activity resulting in a large number of cauline neoformations - buds - devoid of spines and areoles, green - yellowish, with a lumpy, misshapen appearance (Figure 7-5a,5b).

CONCLUSIONS

The current experiment was initiated to determine and compare how the light source, fluorescent tubes or LEDs, and the wavelength, respectively the color of the light (white, blue, yellow, green, red), influence rhizogenesis, caulogenesis and callusogenesis *in vitro* cultures of *R. heliosa*. The results obtained indicate that fluorescent tube light is more suitable for the morphogenesis of *R. heliosa* vitroplants, but the light intensity directly influences the regeneration and organogenic processes. Specifically, in this experiment, it was noticed that the green and red light emitted by LEDs have a positive impact mainly on the rhizogenesis and caulogenesis of *R. heliosa* vitroplants, while the white and yellow light of fluorescent tubes favor caulogenesis and callusogenesis.

These results certify the fact that, in *in vitro* cultures of *R. heliosa*, light is a decisive factor in the regulation of plant growth and development, especially the wavelength involved in photomorphogenic responses. This fact leads to the conclusion that due to the possibility of matching the wavelengths of LEDs with the photoreceptors of vitroplants, it is possible to use them as an alternative and viable source to fluorescent tubes.

REFERENCES

Angulo-Bejarano, PI., Paredes-López, O. (2011) Development of a regeneration protocol through indirect organogenesis in prickly pear cactus [*Opuntia ficus-indica* (L.) Mill]. Scientia Horticulturae, 128, 283–288. DOI: https://doi.org/10.1016/j.scienta.2011.01.030

Armando, C.F., Dias, F.E.C., Fernando, D., Andreote, P., Lacava, M., Teixeira, L., Assumpção, C., Welington, L.A., João, L.A., Itamar Melo, S. (2009) Jurnalul Mondial de Microbiologie și Biotehnologie, 25 (2), 189-195.

Bau, B.W. (2015) A highly efficient *in vitro* propagation protocol for elephant tusk cactus: *Coryphantha elephantidens* (Lem.) Journal of Genetic Engineering and Biotechnology, 13, 215–219. DOI: https://doi.org/10.1016/j.jgeb.2015.07.003

Briggs, W.R., Olney, M.A. (2001) Photoreceptors in plant photomorphogenesis to date, five photochromes, two cryptochrome, one phototropin and one superchrome. Plant Physiology, 125 (1), 85–88. DOI: https://doi.org/10.1104/pp.125.1.85

Budiarto, K. (2010) Spectral quality affects morphogenesis on Anthurium plantlet during *in vitro* culture. Agrivita, Journal of Agricultural Science, 32, 234–240.

Cachiță, C.D., Deliu, C., Tican, R.L., Ardelean, A. (2004) Tratat de biotehnologie vegetală. vol.I, Editura Dacia, Cluj-Napoca, 29-154.

Casas, A., Barbera, G. (2002) Mesoamerican domestication and diffusion. Cacti, Biology and Uses 143, 62. DOI: https://doi.org/10.1525/california/9780520231573.003.0009

Casierra-Posada, F., Matallana-Díaz, Y.A., Zapata-Casierra, E. (2014) Growth of bell pepper plants (*Capsicum annuum*) affected by coloured covers, Gesunde Pflanzen, Journal of Crop Health 66, 4, 149-155.

Cavallaro, V., Avola, G., Fascella, G., Pellegrino, A., Ierna, A. (2023) Effects of Spectral Quality and Light Quantity of LEDs on *In Vitro* Shoot Development and Proliferation of Ananas comosus L. Merr. MDPI, Agronomy, 13, 1072. DOI: https://doi.org/10.3390/agronomy13041072

Chen, J., Henny, R.J., McConnell, D.B. (2002) Development of new foliage plant cultivars. Florida Agricultural Experiment Station Journal Series, No. R-08541.

Cioć, M., Agnieszka, S., Marek, Ż., Andrzej, K., Bożena, P. (2018) LED lighting affects plant growth, morphogenesis and phytochemical contents of *Myrtus communis* L. *in vitro*, Plant Cell, Tissue and Organ Culture,132, 433–447.

Clayton, P.W., Hubstenberg, J.F., Phillips, G.C., Butler–Nance, S.A. (1990) Micropropagation of members of the *Cactaceae* subtribe Cactinae. Journal of the American Society for Horticultural Science, 115, 2, 337 – 343. DOI: https://doi.org/10.21273/JASHS.115.2.337

Martínez-Estrada, E., Caamal-Velázquez, J.H., Morales-Ramos, V., JabínBello J. (2016) Light emitting diodes improve *in vitro* shoot multiplication and growth of *Anthurium andreanum* lind. Propagation of Ornamental Plants, 16, 1.

Estrada-Luna, A.A., Martínez-Hernández, J., Torres-Torres, M.E., Chable-Moreno, F. (2008) *In vitro* micropropagation of the ornamental prickly pear cactus Opuntia lanigera Salm-Dyck and effects of sprayed GA3 after transplantation to *ex vitro* conditions. Scientia Horticulturae, 117,378–385. DOI: https://doi.org/10.1016/j.scienta.2008.05.042

Fang, W., Jao, R.C., Lee, D.H. (2002) Artificial lighting apparatus for young plants using light emitting diodes as light source.; U.S.A., Patent Application Publication; no.: US2002/0154504.

Felipe, A.M., Bordignon, S.R., Rossi, M., Ambrosano, G. (2013) *In vitro* culture of sugarcane exposed to different light sources. Pesquisa Agropecuaria Brasileira, 48 (9), 1303-1307. DOI: https://doi.org/10.1590/S0100-204X2013000900015 (in Portuguese).

Ferreira, C.C.S., Paz, C.D., Souza, J.C., Peixoto, A.R., Rios, L.S., Nascimento, A.R. (2021) Development of the prickly pear cactus *Opuntia stricta* (Haw.) Haw. (*Cactaceae*) *in vitro* in response to the replacement of potassium nitrate for a commercial kno 3 fertilizer. Ciencia Rural, 52, 1–6.

Folta, K.M. (2004) Green light increases early stem elongation rate, antagonizing light-mediated growth inhibition. Plant Physiological Ecology, 135, 1407-1416. DOI: https://doi.org/10.1104/pp.104.038893.

Franklin, K.A., Quail, P.H. (2010) Phytochrome functions in Arabidopsis development, Journal of Experimental Botany, 61, 11-24. DOI: https://doi.org/10.1093/jxb/erp304

Gabryszewska, E., Rudnicki, R. (1995) The influence of light quality on the shoot proliferation and rooting of *Gerbera jamesonii in vitro*. Acta Agrobotanica, 48 (2), 105-111. DOI: https://doi.org/10.5586/aa.1995.021

Garcés, M. (2003) Desarrollo de las primeras etapas de un protocolo de micropropagación para *Eriosyce aurata* (Pfeiffer) Backeberg (*Cactaceae*), una especie en estado de conservación vulnerable endémica de Chile. Tesis Lic. Agr. Santiago, Pontificia Universidad Católica de Chile, Facultad de Agronomia e Ingienería Forestal, 54 p.

Garcıa-Saucedo, P.A., Valdez-Morales, M., Valverde, M.E., Cruz-Hernandez, A., Paredes-Lopez, O. (2005) Regeneration of three Opuntia genotypes used as human food. Plant Cell, Tissue and Organ Culture, 80, 215–219.

Gupta, S.D., Jatothu, B. (2013) Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. Plant Biotechnology Reports, 7 (3), 211-220.

Hahn, E.J., Kozai, T., Paek, K.Y. (2000) Blue and red light-emitting diodes with or without sucrose and ventilation affect *in vitro* growth of *Rehmannia glutinosa* plantlets. Journal of Plant Biology, 43, 247–250. DOI: https://doi.org/10.1007/BF03030425

Heller, R. (1953) Rescherches sur la nutrition minérale des tissus végétaux cultives *in vitro*. Annales des Sciences Naturalles,vol. II, 1-5.

Ht, G.O., Mendoza, A.B., Bocardo, L.E., Quintanilla, J.V., Oviedo, E.C., (2011) Hyperhydricity control of *in vitro* shoots of Turbinicarpus valdezianus (Möller) GL and F. Phyton-International Journal of Experimental Botany, 80(all), 175-179. DOI: https://doi.org/10.32604/phyton.2011.80.175

Jonathan, H.C., Griffing, G.T.O. (2022) Plant photoreceptors and their signaling components in chloroplastic anterograde and retrograde communication, Journal of Experimental Botany, 73 (21), 7126–7138. DOI: https://doi.org/10.1093/jxb/erac220

Kim, H., Goins, G.D., Wheeler, R.M., Sager, J.C. (2004a) Stomatal conductance of lettuce grown under or exposed to different light qualities. Annals of Botany, 94, 691–697. DOI: https://doi.org/10.1093/aob/mch192

Kurilcik, A., Canova, M.R., Dapkuniene, S., Zilinskaite, S., Kurilcik, G., Tamulaitis, G., Duchovskis, P., Zukauskas, A. (2008) *In vitro* culture of Chrysanthemum plantlets using light emitting diodes. Central European Journal of Biology, 3, 161–167. DOI: https://doi.org/10.2478/s11535-008-0006-9

Lema-Rumińska, J., Kulus, D. (2014) Micropropagation of cacti: a review. Haseltonia, 19, 46-63. DOI: https://doi.org/10.2985/026.019.0107

Li, C.X., Xu, Z.G., Dong, R.Q., Chang, S.X., Wang, L.Z., Khalil-Ur-Rehman, M., Tao, J.M. (2017) An RNA-Seq analysis of grape plantlets grown *in vitro* reveals different responses to blue, green, red led light, and white fluorescent light. Frontiers in Plant Science, 8, 78. DOI: https://doi.org/10.3389/fpls.2017.00078

Lin, Y., Li, J., Li, B., He, T., Chun, Z. (2011) Effects of light quality on growth and development of protocorm-like bodies of Dendrobium officinale *in vitro*; Plant Cell, Tissue and Organ Culture, 105, 329-335. DOI: https://doi.org/10.1007/s11240-010-9871-9

Lim, M.J., Murthy, H.N., Lee H.Y., Park, S.Y. (2023) Influence of White, Red, Blue, and Combination of LED Lights on *in vitro* Multiplication of Shoots, Rooting, and Acclimatization of *Gerbera jamesonii* cv. 'Shy Pink' Plants. Agronomy, 13, 2216. DOI: https://doi.org/10.3390/agronomy13092216.

Liu, M., Xu, Z., Yang, Y., Feng, Y. (2011) Effects of different spectral lights on *Oncidium* PLBs induction, proliferation, and plant regeneration, Plant Cell Tissue and Organ Culture, 106 (1), 1-10.

Loberand, B., Arie, A. (2010) Micropropagation of Plants, Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, Volume Set 7.

Mabrouk, A., Abbas, Y., El Goumi, Y., El Maaiden, E., El Kharrassi, Y., Antry-Tazi, S.E. (2021) Optimization of prickly pear cacti (Opuntia spp.) micropropagation using an experimental design method. Journal of microbiology, biotechnology and food sciences, 11 (3), e1577. DOI: https://doi.org/10.15414/jmbfs.1577

Manivannan, A., Soundararajan, P., Gyeong Yoo, P., Wei, H. (2017) Blue and red light-emitting diodes improve the growth and physiology of *in vitro*-grown carnations 'Green Beauty' and 'Purple Beauty', Horticulture, Environment and Biotechnology, 5 (1), 12-20. DOI: https://doi.org/10.1007/s13580-017-0051-2

Martha, M., Murillo-Talavera, M., Pedraza-Santos E., Gutiérrez-Rangel, N., María de las, N.R.M., Lobit, P., Palacios, A.M. (2016) Led light quality and *in vitro* development of *Oncidium tigrinum* and Laeliaautumnalis (Orchidaceae), Agrociencia, 50, nr. 8. DOI: https://doi.org/10.1590/2447-536X.v29i2.2610

Muleo, R., Morini, S., Casano, S. (2001) Photoregulation of growth and branching of plum shoots: Physiological action of two photosystems. *In Vitro* Cellular& Developmental Biolology Plant, 37, 609–617. DOI: https://doi.org/10.1007/s11627-001-0107-x

Murashige, T., Skoog, F., (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15, 473 –497.

Murillo-Talavera, M.M., Pedraza-Santos, M.E., Gutiérrez-Rangel, N., De las Nieves Rodríguez, M. (2016) Led light quality and *in vitro* development of *Oncidium tigrinum* and *Laelia autumnalis* (Orchidaceae), Agrociencia, 50 (8), 1065-1080.

Nhut, D.T., Nam, N.B. (2010) Light emitting diodes (LEDs): an artificial lighting source for biological studies. Proceedings of the 3rd international conference of the development of BME in Vietnam, 11–14.

Nowakowska, K., Kowalczyk, K., Pawełczak, A., Gajc-Wolska, J. (2023) Energy Efficiency of LEDs during Micropropagation of Helleborus 'Molly's White'. Agriculture, 13, 1265. DOI: https://doi.org/10.3390/agriculture13061265

Ortega-Baes, P., Sühring, S., Sajama, J., Sotola, E., Alonso-Pedano, M., Bravo, S., Godínez-Álvarez, H. (2010) Diversity and conservation in the cactus family. In: Ramawat, K.C., ed. Desert plants. Berlin: Springer, pp. 157-173. DOI: https://doi.org/10.1007/978-3-642-02550-1_8

Papafotiou, M., Balots, G., Louka, P., Chronopoulos, J. (2001) *In vitro* plant regeneration of Mammillaria elongata normal and cristate forms. Plant Cell, Tissue and Organ Culture, 65, 163–167.

Pardo, G., Paniagua, P., Aguilar, C.H., Martínez, F.R., Pacheco, A.D., González, C. M., Canseco M.M. (2014) Effects of Light Emitting Diode High Intensity on Growth of Lettuce (Lactuca sativa L.) and Broccoli (Brassica oleracea L.) Seedlings. Annual Research & Review in Biology, 4 (19).

Poudel, P.R., Kataoka, I., Mochioka, R. (2008) Effect of redand blue-light-emitting diodes on growth and morphogenesis of grapes. Plant Cell. Tissue &Organ Culture, 92, 147-153. DOI: https://doi.org/10.1007/s11240-007-9317-1

Pérez, E, Dávila C. (2002) *In vitro* propagation of Pelecyphora aselliformis Ehrenberg, and P. strobiliformis Wedermann (*Cactaceae*). *In vitro* Cellular Development Biology Plant, 38 (1), 73 – 78.

Ramirez-Malagon, R., Aguilar-Ramirez I., Borodanenko, A., Perez-Moreno, L., Barrera-Guerra, J., Nuñez-Palenius, H. (2007) *In vitro* propagation of ten threatened species of Mammillaria (*Cactaceae*). *In Vitro* Cellular and Devolopmental Biology Plant, 43, 660–665. DOI: https://doi.org/10.1079/IVP2001248

Saher, S., Piqueras, A., Hellin, E., Olmos, E. (2004) Hyperhydricity in micropropagated carnation shoots: the role of oxidative stress. Physiology Plant, 120, 152–161.

Schindler, W., Lee, P.G. (2010) Comprehensive conservation planning to protect biodiversity and ecosystem services in Canadian boreal regions under a warming climate and increasing exploitation, Biological Conservation, 143, 7, 1571-1586. DOI: https://doi.org/10.1016/j.biocon.2010.04.003

Souza, L.M., Barbosa, M.R., Zárate-Salazar, J.R., Lozano-Isla, F., Camara, T.R. (2019) Use of meta-Topolin, an unconventional cytokinin in the *in vitro* multiplication of *Opuntia stricta* haw. Biotecnología Vegetal, 19 (2), 85–97.

Toshinari, G., Kazuhiro, F., Kaiyun, G., Kazamitsu, M. (2011) Effects of wave length of LED-light on *in vitro* asymbiotic germination and seedling growth of *Bletilla ochracea* Schltr. (Orchidaceae); Plant Biotehnology, 28 (4), 397-400. DOI: https://doi.org/10.5511/plantbiotechnology.11.0524a

Wellens, R. (2003) Succulent tissue culture. Cultivar, 5, 21, 9-18.

Werbrouck, S., Buyle, H., Geelen, D., Van Labeke, M.C. (2012) Effect of red-, far-red- and blue-light-emitting diodes on *in vitro* growth of *Ficus benjamina*. Acta Horticulturae, 961, 533–538.

Winson, K.W.S., Chew, B.L., Sathasivam, K., Subramaniam, S. (2021) Effect of amino acid supplementation, elicitation and LEDs on *Hylocereus costaricensis* callus culture for the enhancement of betalain pigments. Scientia Horticulturae, 289, 110459. DOI: https://doi.org/10.1016/j.scienta.2021.110459

Winslow, R.B. (2002) Plant photoreceptors: proteins that perceive information vital for plant development from the light environment, The Urban Wildrands group and Ecological Consequences of Artificial Night Lighting, Department of Plant Biology, Carnegie Institution of Washington, USA, 260 p.

Yeh, H., Chung, J.P. (2009) High-brightness LEDs-energy efficient lighting sources and their potential in indoor plant cultivation. Renewable and Sustainable Energy Reviews, 13, 2175–2180. DOI: https://doi.org/10.1016/j.rser.2009.01.027